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(54) Title: HIGH MOLECULAR WEIGHT SURFACE PROTEINES OF NON-TYPEABLE HAEMOPHILUS

(57) Abstract

High molecular weight surface proteins of non-typeable *Haemophilus influenzae* which exhibit immunogenic properties and genes encoding the same are described. Specifically, genes coding for two immunodominant high molecular weight proteins, HMW1 and HMW2, have been cloned, expressed and sequenced, while genes coding for high molecular proteins HMW3 and HMW4 have been cloned, expressed and partially sequenced.

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TITLE OF INVENTION

HIGH MOLECULAR WEIGHT SURFACE PROTEINS OF NON-TYPEABLE HAEMOPHILUS

FIELD OF INVENTION

This invention relates to high molecular weight proteins of non-typeable haemophilus.

BACKGROUND TO THE INVENTION

Non-typeable Haemophilus influenzae are non-encapsulated organisms that are defined by their lack of reactivity with antisera against known H. influenzae capsular antigens.

These organisms commonly inhabit the upper respiratory tract of humans and are frequently responsible for infections, such as otitis media, sinusitis, conjunctivitis, bronchitis and pneumonia. Since these organisms do not have a polysaccharide capsule, they are not controlled by the present Haemophilus influenzae type b (Hib) vaccines, which are directed towards Hib bacterial capsular polysaccharides. The non-typeable strains, however, do produce surface antigens that can elicit bactericidal antibodies. Two of the major outer membrane proteins, P2 and P6, have been identified as targets of human serum bactericidal activity. However, it has been shown that the P2 protein sequence is variable, in particular in the non-typeable Haemophilus strains. Thus, a P2-based vaccine would not protect against all strains of the organism.

There have previously been identified by Barenkamp et al (Pediatr. Infect. Dis. J., 9:333-339, 1990) a group of high-molecular-weight (HMW) proteins that appeared to be major targets of antibodies present in human convalescent sera. Examination of a series of middle ear isolates revealed the presence of one or two such proteins in most strains. However, prior to the present invention, the structures of these proteins were unknown as were pure isolates of such proteins.

SUMMARY OF INVENTION

The inventors, in an effort to further characterize the high molecular weight (HMW) Haemophilus proteins, have cloned, expressed and sequenced the genes coding for two immunodominant HMW proteins (designated HMW1 and HMW2) from a prototype non-typeable Haemophilus strain and have cloned, expressed and almost completely sequenced the genes coding for two additional immunodominant HMW proteins (designated HMW3 and HMW4) from another non-typeable Haemophilus strain.

In accordance with one aspect of the present invention, therefore, there is provided an isolated and purified gene coding for a high molecular weight protein of a non-typeable Haemophilus strain, particularly a gene coding for protein HMW1, HMW2, HMW3 or HMW4, as well as any variant or fragment of such protein which retains the immunological ability to protect against disease caused by a non-typeable Haemophilus strain. In another aspect, the invention provides a high molecular weight protein of non-typeable Haemophilus influenzae which is encoded by these genes.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 is a DNA sequence of a gene coding for protein HMW1 (SEQ ID NO: 1);

Figure 2 is a derived amino acid sequence of protein HMW1 (SEQ ID NO: 2);

Figure 3 is a DNA sequence of a gene coding for protein HMW2 (SEQ ID NO: 3);

Figure 4 is a derived amino acid sequence of HMW2 (SEQ ID NO: 4);

Figure 5A shows restriction maps of representative recombinant phages which contained the HMW1 or HMW2 structural genes, the locations of the structural genes being indicated by the shaded bars;

Figure 5B shows the restriction map of the T7 expression vector pT7-7;

Figure 6 contains the DNA sequence of a gene cluster for the hmw1 gene (SEQ ID NO: 5), comprising nucleotides 351 to 4958 (ORF a) (as in Figure 1), as well as two additional downstream genes in the 3′ flanking region, comprising ORFs b, nucleotides 5114-6748 and c nucleotides 7062-9011;

Figure 7 contains the DNA sequence of a gene cluster for the hmw2 gene (SEQ ID NO: 6), comprising nucleotides 792 to 5222 (ORF a) (as in Figure 3), as well as two additional downstream genes in the 3′ flanking region, comprising ORFs b, nucleotides 5375-7009, and c, nucleotides 7249-9198;

Figure 8 is a partial DNA sequence of a gene coding for protein HMW3 (SEQ ID NO: 7);

Figure 9 is a partial DNA sequence of a gene coding for protein HMW4 (SEQ ID NO: 8); and

Figure 10 is a comparison table for the derived amino acid sequence for proteins HMW1, HMW2, HMW3 and HMW4.

GENERAL DESCRIPTION OF INVENTION

The DNA sequences of the genes coding for HMW1 and HMW2, shown in Figures 1 and 3 respectively, were shown to be about 80% identical, with the first 1259 base pairs of the genes being identical. The derived amino acid sequences of the two HMW proteins, shown in Figures 2 and 4 respectively, are about 70% identical. Furthermore, the encoded proteins are antigenically related to the filamentous hemagglutinin surface protein of Bordetella pertussis. A monoclonal antibody prepared against filamentous hemagglutinin (FHA) of Bordetella pertussis was found to recognize both of the high molecular weight proteins. This data suggests that the HMW and FHA proteins may serve similar biological functions. The derived amino acid sequences of the HMW1 and HMW2 proteins show sequence similarity to that for the FHA protein. It has further been shown that these

antigenically-related proteins are produced by the majority of the non-typeable strains of Haemophilus. Antisera raised against the protein expressed by the HMW1 gene recognizes both the HMW2 protein and the B. pertussis FHA. The present invention includes an isolated and purified high molecular weight protein of non-typeable haemophilus which is antigenically related to the B. pertussis FHA, which may be obtained from natural sources or produced recombinantly.

A phage genomic library of a known strain of non-typeable Haemophilus was prepared by standard methods and the library was screened for clones expressing high molecular weight proteins, using a high titre antiserum against HMW's. A number of strongly reactive DNA clones were plaque-purified and sub-cloned into a T7 expression plasmid. It was found that they all expressed either one or the other of the two high-molecular-weight proteins designated HMW1 and HMW2, with apparent molecular weights of 125 and 120 kDa, respectively, encoded by open reading frames of 4.6 kb and 4.4 kb, respectively.

Representative clones expressing either HMW1 or HMW2 were further characterized and the genes isolated, purified and sequenced. The DNA sequence of HMW1 is shown in Figure 1 and the corresponding derived amino acid sequence in Figure 2. Similarly, the DNA sequence of HMW2 is shown in Figure 3 and the corresponding derived amino acid sequence in Figure 4. Partial purification of the isolated proteins and N-terminal sequence analysis indicated that the expressed proteins are truncated since their sequence starts at residue number 442 of both full length HMW1 and HMW2 gene products.

Subcloning studies with respect to the hmw1 and hmw2 genes indicated that correct processing of the HMW proteins required the products of additional downstream genes. It has been found that both the hmw1 and hmw2 genes are flanked by two additional downstream open

reading frames (ORFs), designated b and c, respectively, (see Figures 6 and 7).

The b ORFs are 1635 bp in length, extending from nucleotides 5114 to 6748 in the case of hmw1 and nucleotides 5375 to 7009 in the case of hmw2, with their derived amino acid sequences 99% identical. The derived amino acid sequences demonstrate similarity with the derived amino acid sequences of two genes which encode proteins required for secretion and activation of hemolysins of P. mirabilis and S. marcescens.

The c ORFs are 1950 bp in length, extending from nucleotides 7062 to 9011 in the case of hmw1 and nucleotides 7249 to 9198 in the case of hmw2, with their derived amino acid sequences 96% identical. The hmw1 c ORF is preceded by a series of 9 bp direct tandem repeats. In plasmid subclones, interruption of the hmw1 b or c ORF results in defective processing and secretion of the hmw1 structural gene product.

The two high molecular weight proteins have been isolated and purified and shown to be partially protective against otitis media in chinchillas and to function as adhesins. These results indicate the potential for use of such high molecular proteins and structurally-related proteins of other non-typeable strains of Haemophilus influenzae as components in non-typeable Haemophilus influenzae vaccines.

Since the proteins provided herein are good cross-reactive antigens and are present in the majority of non-typeable Haemophilus strains, it is evident that these HMW proteins may become integral constituents of a universal Haemophilus vaccine. Indeed, these proteins may be used not only as protective antigens against otitis, sinusitis and bronchitis caused by the non-typeable Haemophilus strains, but also may be used as carriers for the protective Hib polysaccharides in a conjugate vaccine against meningitis. The proteins also

may be used as carriers for other antigens, haptens and polysaccharides from other organisms, so as to induce immunity to such antigens, haptens and polysaccharides.

The nucleotide sequences encoding two high molecular weight proteins of a different non-typeable Haemophilus strain (designated HMW3 and HMW4) have been largely elucidated, and are presented in Figures 8 and 9. HMW3 has an apparent molecular weight of 125 kDa while HMW4 has an apparent molecular weight of 123 kDa. These high molecular weight proteins are antigenically related to the HMW1 and HMW2 proteins and to FHA. Sequence analysis of HMW3 is approximately 85% complete and of HMW4 95% complete, with short stretches at the 5'-ends of each gene remaining to be sequenced.

Figure 10 contains a multiple sequence comparison of the derived amino acid sequences for the four high molecular weight proteins identified herein. As may be seen from this comparison, stretches of identical peptide sequence may be found throughout the length of the comparison, with HMW3 more closely resembling HMW1 and HMW4 more closely resembling HMW2. This information is highly suggestive of a considerable sequence homology between high molecular weight proteins from various non-typeable Haemophilus strains.

In addition, mutants of non-typeable H. influenzae strains that are deficient in expression of HMW1 or HMW2 or both have been constructed and examined for their capacity to adhere to cultured human epithelial cells. The hmw1 and hmw2 gene clusters have been expressed in E. coli and have been examined for in vitro adherence. The results of such experimentation demonstrate that both HMW1 and HMW2 mediate attachment and hence are adhesins and that this function is present even in the absence of other H. influenzae surface structures.

With the isolation and purification of the high molecular weight proteins, the inventors are able to

determine the major protective epitopes by conventional epitope mapping and synthesize peptides corresponding to these determinants to be incorporated in fully synthetic or recombinant vaccines. Accordingly, the invention also comprises a synthetic peptide having an amino acid sequence corresponding to at least one protective epitope of a high molecular weight protein of a non-typeable Haemophilus influenzae. Such peptides are of varying length that constitute portions of the high-molecular-weight proteins, that can be used to induce immunity, either directly or as part of a conjugate, against the relative organisms and thus constitute vaccines for protection against the corresponding diseases.

The present invention also provides any variant or fragment of the proteins that retains the potential immunological ability to protect against disease caused by non-typeable Haemophilus strains. The variants may be constructed by partial deletions or mutations of the genes and expression of the resulting modified genes to give the protein variations.

EXAMPLES

Example 1:

Non-typeable H.influenzae strains 5 and 12 were isolated in pure culture from the middle ear fluid of children with acute otitis media. Chromosomal DNA from strain 12, providing genes encoding proteins HMW1 and HMW2, was prepared by preparing Sau3A partial restriction digests of chromosomal DNA and fractionating on sucrose gradients. Fractions containing DNA fragments in the 9 to 20 kbp range were pooled and a library was prepared by ligation into λEMBL3 arms. Ligation mixtures were packaged in vitro and plate-amplified in a P2 lysogen of E. coli LE392.

For plasmid subcloning studies, DNA from a representative recombinant phage was subcloned into the

T7 expression plasmid pT7-7, containing the T7 RNA polymerase promoter Φ10, a ribosome-binding site and the translational start site for the T7 gene 10 protein upstream from a multiple cloning site (see Figure 5B).

DNA sequence analysis was performed by the dideoxy method and both strands of the HMW1 gene and a single strand of the HMW2 gene were sequenced.

Western immunoblot analysis was performed to identify the recombinant proteins being produced by reactive phage clones. Phage lysates grown in LE392 cells or plaques picked directly from a lawn of LE392 cells on YT plates were solubilized in gel electrophoresis sample buffer prior to electrophoresis. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was performed on 7.5% or 11% polyacrylamide modified Laemmli gels. After transfer of the proteins to nitrocellulose sheets, the sheets were probed sequentially with an E. coli-absorbed human serum sample containing high-titer antibody to the high-molecular-weight proteins and then with alkaline phosphatase-conjugated goat anti-human immunoglobulin G (IgG) second antibody. Sera from healthy adults contains high-titer antibody directed against surface-exposed high-molecular-weight proteins of non-typeable H. influenzae. One such serum sample was used as the screening antiserum after having been extensively absorbed with LE392 cells.

To identify recombinant proteins being produced by E. coli transformed with recombinant plasmids, the plasmids of interest were used to transform E. coli BL21 (DE3)/pLysS. The transformed strains were grown to an A_{600} of 0.5 in L broth containing 50 μg of ampicillin per ml. IPTG was then added to 1 mM. One hour later, cells were harvested, and a sonicate of the cells was prepared. The protein concentrations of the samples were determined by the bicinchoninic acid method. Cell sonicates

containing 100 μg of total protein were solubilized in electrophoresis sample buffer, subjected to SDS-polyacrylamide gel electrophoresis, and transferred to nitrocellulose. The nitrocellulose was then probed sequentially with the E. coli-absorbed adult serum sample and then with alkaline phosphatase-conjugated goat anti-human IgG second antibody.

Western immunoblot analysis also was performed to determine whether homologous and heterologous non-typeable H. influenzae strains expressed high-molecular-weight proteins antigenically related to the protein encoded by the cloned HMW1 gene (rHMW1). Cell sonicates of bacterial cells were solubilized in electrophoresis sample buffer, subjected to SDS-polyacrylamide gel electrophoresis, and transferred to nitrocellulose. Nitrocellulose was probed sequentially with polyclonal rabbit rHMW1 antiserum and then with alkaline phosphatase-conjugated goat anti-rabbit IgG second antibody.

Finally, Western immunoblot analysis was performed to determine whether non-typeable Haemophilus strains expressed proteins antigenically related to the filamentous hemagglutinin protein of Bordetella pertussis. Monoclonal antibody X3C, a murine immunoglobulin G (IgG) antibody which recognizes filamentous hemagglutinin, was used to probe cell sonicates by Western blot. An alkaline phosphatase-conjugated goat anti-mouse IgG second antibody was used for detection.

To generate recombinant protein antiserum, E. coli BL21(DE3)/pLysS was transformed with pHMW1-4, and expression of recombinant protein was induced with IPTG, as described above. A cell sonicate of the bacterial cells was prepared and separated into a supernatant and pellet fraction by centrifugation at 10,000 x g for 30 min. The recombinant protein fractionated with the

pellet fraction. A rabbit was subcutaneously immunized on biweekly schedule with 1 mg of protein from the pellet fraction, the first dose given with Freund's complete adjuvant and subsequent doses with Freund's incomplete adjuvant. Following the fourth injection, the rabbit was bled. Prior to use in the Western blot assay, the antiserum was absorbed extensively with sonicates of the host E. coli strain transformed with cloning vector alone.

To assess the sharing of antigenic determinants between HMW1 and filamentous hemagglutinin, enzyme-linked immunosorbent assay (ELISA) plates (Costar, Cambridge, Mass.) were coated with 60 µl of a 4-ug/ml solution of filamentous hemagglutinin in Dulbecco's phosphate-buffered saline per well for 2 h at room temperature. Wells were blocked for 1 h with 1% bovine serum albumin in Dulbecco's phosphate-buffered saline prior to addition of serum dilutions. rHMW1 antiserum was serially diluted in 0.1% Brij (Sigma, St. Louis, Mo.) in Dulbecco's phosphate-buffered saline and incubated for 3 h at room temperature. After being washed, the plates were incubated with peroxidase-conjugated goat anti-rabbit lgG antibody (Bio-Rad) for 2 h at room temperature and subsequently developed with 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma) at a concentration of 0.54 in mg/ml in 0.1 M sodium citrate buffer, pH 4.2, containing 0.03% H_2O_2. Absorbances were read on an automated ELISA reader.

Recombinant phage expressing HMW1 or HMW2 were recovered as follows. The non-typeable H. influenzae strain 12 genomic library was screened for clones expressing high-molecular-weight proteins with an E. coli-absorbed human serum sample containing a high titer of antibodies directed against the high-molecular-weight proteins.

Numerous strongly reactive clones were identified along with more weakly reactive ones. Twenty strongly reactive clones were plaque-purified and examined by Western blot for expression of recombinant proteins. Each of the strongly reactive clones expressed one of two types of high-molecular-weight proteins, designated HMW1 and HMW2. The major immunoreactive protein bands in the HMW1 and HMW2 lysates migrated with apparent molecular masses of 125 and 120 kDa, respectively. In addition to the major bands, each lysate contained minor protein bands of higher apparent molecular weight. Protein bands seen in the HMW2 lysates at molecular masses of less than 120 kDa were not regularly observed and presumably represent proteolytic degradation products. Lysates of LE392 infected with the λEMBL3 cloning vector alone were non-reactive when immunologically screened with the same serum sample. Thus, the observed activity was not due to cross-reactive E. coli proteins or λEMBL3-encoded proteins. Furthermore, the recombinant proteins were not simply binding immunoglobulin nonspecifically, since the proteins were not reactive with the goat anti-human IgG conjugate alone, with normal rabbit sera, or with serum from a number of healthy young infants.

Representative clones expressing either the HMW1 or HMW2 recombinant proteins were characterized further. The restriction maps of the two phage types were different from each other, including the regions encoding the HMW1 and HMW2 structural genes. Figure 5A shows restriction maps of representative recombinant phage which contained the HMW1 or HMW2 structural genes. The locations of the structural genes are indicated by the shaded bars.

HMW1 plasmid subclones were constructed by using the T7 expression plasmid T7-7 (Fig. 5A and B). HMW2 plasmid subclones also were constructed, and the results with

these latter subclones were similar to those observed with the HMW1 constructs.

The approximate location and direction of transcription of the HMW1 structure gene were initially determined by using plasmid pHMW1 (Fig. 5A). This plasmid was constructed by inserting the 8.5-kb BamHI-SalI fragment from λHMW1 into BamHI- and SalI-cut pT7-7. E. coli transformed with pHMW1 expressed an immunoreactive recombinant protein with an apparent molecular mass of 115 kDa, which was strongly inducible with IPTG. This protein was significantly smaller than the 125-kDa major protein expressed by the parent phage, indicating that it either was being expressed as a fusion protein or was truncated at the carboxy terminus.

To more precisely localize the 3′ end of the structural gene, additional plasmids were constructed with progressive deletions from the 3′ end of the pHMW1 construct. Plasmid pHMW1-1 was constructed by digestion of pHMW1 with PstI, isolation of the resulting 8.8-kb fragment, and religation. Plasmid pHMW1-2 was constructed by digestion of pHMW1 with HindIII, isolation of the resulting 7.5-kb fragment, and religation. E. coli transformed with either plasmid pHMW1-1 or pHMW1-2 also expressed an immunoreactive recombinant protein with an apparent molecular mass of 115 kDa. These results indicated that the 3′ end of the structural gene was 5′ of the HindIII site.

To more precisely localize the 5′ end of the gene, plasmids pHMW1-4 and pHMW1-7 were constructed. Plasmid pHMW1-4 was constructed by cloning the 5.1-kb BamHI-HindIII fragment from λHMW1 into a pT7-7-derived plasmid containing the upstream 3.8-kb EcoRI-BamHi fragment. E. coli transformed with pHMW1-4 expressed an immunoreactive protein with an apparent molecular mass of approximately 160 kDa. Although protein production was inducible with IPTG, the levels of protein production in these

transformants were substantially lower than those with the pHMW1-2 transformants described above. Plasmid pHMW1-7 was constructed by digesting pHMW1-4 with NdeI and SpeI. The 9.0-kbp fragment generated by this double digestion was isolated, blunt ended, and religated. E. coli transformed with pHMW1-7 also expressed an immunoreactive protein with an apparent molecular mass of 160 kDa, a protein identical in size to that expressed by the pHMW1-4 transformants. The result indicated that the initiation codon for the HMW1 structural gene was 3′ of the SpeI site. DNA sequence analysis confirmed this conclusion.

As noted above, the λHMW1 phage clones expressed a major immunoreactive band of 125 kDa, whereas the HMW1 plasmid clones pHMW1-4 and pHMW1-7, which contained what was believed to be the full-length gene, expressed an immunoreactive protein of approximately 160 kDa. This size discrepancy was disconcerting. One possible explanation was that an additional gene or genes necessary for correct processing of the HMW1 gene product were deleted in the process of subcloning. To address this possibility, plasmid pHMW1-14 was constructed. This construct was generated by digesting pHMW1 with NdeI and MluI and inserting the 7.6-kbp NdeI-MluI fragment isolated from pHMW1-4. Such a construct would contain the full-length HMW1 gene as well as the DNA 3′ of the HMW1 gene which was present in the original HMW1 phage. E. coli transformed with this plasmid expressed major immunoreactive proteins with apparent molecular masses of 125 and 160 kDa as well as additional degradation products. The 125- and 160-kDa bands were identical to the major and minor immunoreactive bands detected in the HMW1 phage lysates. Interestingly, the pHMW1-14 construct also expressed significant amounts of protein in the uninduced condition, a situation not observed with the earlier constructs.

The relationship between the 125- and 160-kDa proteins remains somewhat unclear. Sequence analysis, described below, reveals that the HMW1 gene would be predicted to encode a protein of 159 kDa. It is believed that the 160-kDa protein is a precursor form of the mature 125-kDa protein, with the conversion from one protein to the other being dependent on the products of the two downstream genes.

Sequence analysis of the HMW1 gene (Figure 1) revealed a 4,608-bp open reading frame (ORF), beginning with an ATG codon at nucleotide 351 and ending with a TAG stop codon at nucleotide 4959. A putative ribosome-binding site with the sequence AGGAG begins 10 bp upstream of the putative initiation codon. Five other in-frame ATG codons are located within 250 bp of the beginning of the ORF, but none of these is preceded by a typical ribosome-binding site. The 5′-flanking region of the ORF contains a series of direct tandem repeats, with the 7-bp sequence ATCTTTC repeated 16 times. These tandem repeats stop 100 bp 5′ of the putative initiation codon. An 8-bp inverted repeat characteristic of a rho-independent transcriptional terminator is present, beginning at nucleotide 4983, 25 bp 3′ of the presumed translational stop. Multiple termination codons are present in all three reading frames both upstream and downstream of the ORF. The derived amino acid sequence of the protein encoded by the HMW1 gene (Figure 2) has a molecular weight of 159,000, in good agreement with the apparent molecular weights of the proteins expressed by the HMW1-4 and HMW1-7 transformants. The derived amino acid sequence of the amino terminus does not demonstrate the characteristics of a typical signal sequence. The BamHI site used in generation of pHMW1 comprises bp 1743 through 1748 of the nucleotide sequence. The ORF downstream of the BamHI site would be predicted to encode a protein of 111 kDa, in good agreement with the 115 kDa

estimated for the apparent molecular mass of the pHMW1-encoded fusion protein.

The sequence of the HMW2 gene (Figure 3) consists of a 4,431-bp ORF, beginning with an ATG codon at nucleotide 352 and ending with a TAG stop codon at nucleotide 4783. The first 1,259 bp of the ORF of the HMW2 gene are identical to those of the HMW1 gene. Thereafter, the sequences begin to diverge but are 80% identical overall. With the exception of a single base addition at nucleotide 93 of the HMW2 sequence, the 5′-flanking regions of the HMW1 and HMW2 genes are identical for 310 bp upstream from the respective initiation codons. Thus, the HMW2 gene is preceded by the same set of tandem repeats and the same putative ribosome-binding site which lies 5′ of the HMW1 gene. A putative transcriptional terminator identical to that identified 3′ of the HMW1 ORF is noted, beginning at nucleotide 4804. The discrepancy in the lengths of the two genes is principally accounted for by a 186-bp gap in the HMW2 sequence, beginning at nucleotide position 3839. The derived amino acid sequence of the protein encoded by the HMW2 gene (Figure 4) has a molecular weight of 155,000 and is 71% identical with the derived amino acid sequence of the HMW1 gene.

The derived amino acid sequences of both the HMW1 and HMW2 genes (Figures 2 and 4) demonstrated sequence similarity with the derived amino acid sequence of filamentous hemagglutinin of Bordetella pertussis, a surface-associated protein of this organism. The initial and optimized TFASTA scores for the HMW1-filamentous hemagglutinin sequence comparison were 87 and 186, respectively, with a word size of 2. The z score for the comparison was 45.8. The initial and optimized TFASTA scores for the HMW2-filamentous hemagglutinin sequence comparison were 68 and 196, respectively. The z score for the latter comparison was 48.7. The magnitudes of

the initial and optimized TFASTA scores and the z scores suggested that a biologically significant relationship existed between the HMW1 and HMW2 gene products and filamentous hemagglutinin. When the derived amino acid sequences of HMW1, HMW2, and filamentous hemagglutinin genes were aligned and compared, the similarities were most notable at the amino-terminal ends of the three sequences. Twelve of the first 22 amino acids in the predicted peptide sequences were identical. In additional, the sequences demonstrated a common five-amino-acid stretch, Asn-Pro-Asn-Gly-Ile, and several shorter stretches of sequence identity within the first 200 amino acids.

Example 2:

To further explore the HMW1-filamentous hemagglutinin relationship, the ability of antiserum prepared against the HMW1-4 recombinant protein (rHMW1) to recognize purified filamentous hemagglutinin was assessed. The rHMW1 antiserum demonstrated ELISA reactivity with filamentous hemagglutinin in a dose-dependent manner. Preimmune rabbit serum had minimal reactivity in this assay. The rHMW1 antiserum also was examined in a Western blot assay and demonstrated weak but positive reactivity with purified filamentous hemagglutinin in this system also.

To identify the native Haemophilus protein corresponding to the HMW1 gene product and to determine the extent to which proteins antigenically related to the HMW1 cloned gene product were common among other non-typeable H. influenzae strains, a panel of Haemophilus strains was screened by Western blot with the rHMW1 antiserum. The antiserum recognized both a 125- and a 120-kDa protein band in the homologous strain 12, the putative mature protein products of the HMW1 and HMW2 genes, respectively.

When used to screen heterologous non-typeable H. influenzae strains, rHMW1 antiserum recognized high-molecular-weight proteins in 75% of 125 epidemiologically unrelated strains. In general, the antiserum reacted with one or two protein bands in the 100- to 150-kDa range in each of the heterologous strains in a pattern similar but not identical to that seen in the homologous strain.

Monoclonal antibody X3C is a murine IgG antibody directed against the filamentous hemagglutinin protein of B. pertussis. This antibody can inhibit the binding of B. pertussis cells to Chinese hamster ovary cells and HeLa cells in culture and will inhibit hemagglutination of erythrocytes by purified filamentous hemagglutinin. A Western blot assay was performed in which this monoclonal antibody was screened against the same panel of non-typeable H. influenzae strains discussed above. Monoclonal antibody X3C recognized both the high-molecular-weight proteins in non-typeable H. influenzae strain 12 which were recognized by the recombinant-protein antiserum. In addition, the monoclonal antibody recognized protein bands in a subset of heterologous non-typeable H. influenzae strains which were identical to those recognized by the recombinant-protein antiserum. On occasion, the filamentous hemagglutinin monoclonal antibody appeared to recognize only one of the two bands which had been recognized by the recombinant-protein antiserum. Overall, monoclonal antibody X3C recognized high-molecular-weight protein bands identical to those recognized by the rHMW1 antiserum in approximately 35% of our collection of non-typeable H. influenzae strains.

Example 3:

Mutants deficient in expression of HMW1, MW2 or both proteins were constructed to examine the role of these proteins in bacterial adherence. The following strategy was employed. pHMW1-14 (see Example 1, Figure 5A) was

digested with BamHI and then ligated to a kanamycin cassette isolated on a 1.3-kb BamHl fragment from pUC4K. The resultant plasmid (pHMW1-17) was linearized by digestion with XbaI and transformed into non-typeable H. influenzae strain 12, followed by selection for kanamycin resistant colonies. Southern analysis of a series of these colonies demonstrated two populations of transformants, one with an insertion in the HMW1 structural gene and the other with an insertion in the HMW2 structural gene. One mutant from each of these classes was selected for further studies.

Mutants deficient in expression of both proteins were recovered using the following protocol. After deletion of the 2.1-kb fragment of DNA between two EcoRI sites spanning the 3'-portion of the HMW1 structural gene in pHMW-15, the kanamycin cassette from pUC4K was inserted as a 1.3-kb EcoRl fragment. The resulting plasmid (pHMW1-16) was linearized by digestion with XbaI and transformed into strain 12, followed again by selection for kanamycin resistant colonies. Southern analysis of a representative sampling of these colonies demonstrated that in seven of eight cases, insertion into both the HMW1 and HMW2 loci had occurred. One such mutant was selected for further studies.

To confirm the intended phenotypes, the mutant strains were examined by Western blot analysis with a polyclonal antiserum against recombinant HMW1 protein. The parental strain expressed both the 125-kD HMW1 and the 120-kD HMW2 protein. In contrast, the HMW2$^-$ mutant failed to express the 120-kD protein, and the HMW1 mutant failed to express the 125-kD protein. The double mutant lacked expression of either protein. On the basis of whole cell lysates, outer membrane profiles, and colony morphology, the wild type strain and the mutants were otherwise identical with one another. Transmission

electron microscopy demonstrated that none of the four strains expressed pili.

The capacity of wild type strain 12 to adhere to Chang epithelial cells was examined. In such assays, bacteria were inoculated into broth and allowed to grow to a density of ~2 x 10^9 cfu/ml. Approximately 2 x 10^7 cfu were inoculated onto epithelial cell monolayers, and plates were gently centrifuged at 165 x g for 5 minutes to facilitate contact between bacteria and the epithelial surface. After incubation for 30 minutes at 37°C in 5% CO_2, monolayers were rinsed 5 times with PBS to remove nonadherent organisms and were treated with trypsin-EDTA (0.05% trypsin, 0.5% EDTA) in PBS to release them from the plastic support. Well contents were agitated, and dilutions were plated on solid medium to yield the number of adherent bacteria per monolayer. Percent adherence was calculated by dividing the number of adherent cfu per monolayer by the number of inoculated cfu.

As depicted in Table 1 below (the Tables appear at the end of the descriptive text), this strain adhered quite efficiently, with nearly 90% of the inoculum binding to the monolayer. Adherence by the mutant expressing HMW1 but not HMW2 (HMW2$^-$) was also quite efficient and comparable to that by the wild type strain. In contrast, attachment by the strain expressing HMW2 but deficient in expression of HMW1 (HMW1$^-$) was decreased about 15-fold relative to the wild type. Adherence by the double mutant (HMW1$^-$/HMW2$^-$) was decreased even further, approximately 50-fold compared with the wild type and approximately 3-fold compared with the HMW1 mutant. Considered together, these results suggest that both the HMW1 protein and the, HMW2 protein influence attachment to Chang epithelial cells. Interestingly, optimal adherence to this cell line appears to require HMW1 but not HMW2.

Example 4:

Using the plasmids pHMW1-16 and pHMW1-17 (see Example 3) and following a scheme similar to that employed with strain 12 as described in Example 3, three non-typeable Haemophilus strain 5 mutants were isolated, including one with the kanamycin gene inserted into the hmw1-like (designated hmw3) locus, a second with an insertion in the hmw2-like (designated hmw4) locus, and a third with insertions in both loci. As predicted, Western immunoblot analysis demonstrated that the mutant with insertion of the kanamycin cassette into the hmw1-like locus had lost expression of the HMW3 125-kD protein, while the mutant with insertion into the hmw2-like locus failed to express the HMW4 123-kD protein. The mutant with a double insertion was unable to express either of the high molecular weight proteins.

As shown in Table 1 below, wild type strain 5 demonstrated high level adherence, with almost 80% of the inoculum adhering per monolayer. Adherence by the mutant deficient in expression of the HMW2-like protein was also quite high. In contrast, adherence by the mutant unable to express the, HMW1-like protein was reduced about 5-fold relative to the wild type, and attachment by the double mutant was diminished even further (approximately 25-fold). Examination of Giemsa-stained samples confirmed these observations (not shown). Thus, the results with strain 5 corroborate the findings with strain 12 and the HMW1 and HMW2 proteins.

Example 5:

To confirm an adherence function for the HMW1 and HMW2 proteins and to examine the effect of HMW1 and HMW2 independently of other H. influenzae surface structures, the hmw1 and the hmw2 gene clusters were introduced into E. coli DH5α, using plasmids pHMW1-14 and pHMW2-21, respectively. As a control, the cloning vector, pT7-7, was also transformed into E. coli DH5α. Western blot

analysis demonstrated that E. coli DH5α containing the hmw1 genes expressed a 125 kDa protein, while the same strain harboring the hmw2 genes expressed a 120-kDa protein. E. coli DH5α containing pT7-7 failed to react with antiserum against recombinant HMW1. Transmission electron microscopy revealed no pili or other surface appendages on any of the E. coli strains.

Adherence by the E. coli strains was quantitated and compared with adherence by wild type non-typeable H. influenzae strain 12. As shown in Table 2 below, adherence by E. coli DH5α containing vector alone was less than 1% of that for strain 12. In contrast, E. coli DH5α harboring the hmw1 gene cluster demonstrated adherence levels comparable to those for strain 12. Adherence by E. coli DH5α containing the hmw2 genes was approximately 6-fold lower than attachment by strain 12 but was increased 20-fold over adherence by E. coli DH5α with pT7-7 alone. These results indicate that the HMW1 and HMW2 proteins are capable of independently mediating attachment to Chang conjunctival cells. These results are consistent with the results with the H. influenzae mutants reported in Examples 3 and 4, providing further evidence that, with Chang epithelial cells, HMW1 is a more efficient adhesin than is HMW2.

Experiments with E. coli HB101 harboring pT7-7, pHMW1-14, or pHMW2-21 confirmed the results obtained with the DH5α derivatives (see Table 2).

Example 6:

HMW1 and HMW2 were isolated and purified from non-typeable H. influenzae (NTHI) strain 12 in the following manner. Non-typeable Haemophilus bacteria from frozen stock culture were streaked onto a chocolate plate and grown overnight at 37°C in an incubator with 5% CO_2. 50ml starter culture of brain heart infusion (BHI) broth, supplemented with 10 μg/ml each of hemin and NAD was inoculated with growth on chocolate plate. The starter

culture was grown until the optical density (O.D. - 600nm) reached 0.6 to 0.8 and then the bacteria in the starter culture was used to inoculate six 500 ml flasks of supplemented BHI using 8 to 10 ml per flask. The bacteria were grown in 500 ml flasks for an additional 5 to 6 hours at which time the O.D. was 1.5 or greater. Cultures were centrifuged at 10,000 rpm for 10 minutes.

Bacterial pellets were resuspended in a total volume of 250 ml of an extraction solution comprising 0.5 M NaCl, 0.01 M Na_2EDTA, 0.01 M Tris 50 μM 1,10-phenanthroline, pH 7.5. The cells were not sonicated or otherwise disrupted. The resuspended cells were allowed to sit on ice at 0°C for 60 minutes. The resuspended cells were centrifuged at 10,000 rpm for 10 minutes at 4°C to remove the majority of intact cells and cellular debris. The supernatant was collected and centrifuged at 100,000 xg for 60 minutes at 4°C. The supernatant again was collected and dialyzed overnight at 4°C against 0.01 M sodium phosphate, pH 6.0.

The sample was centrifuged at 10,000 rpm for 10 minutes at 4°C to remove insoluble debris precipitated from solution during dialysis. The supernatant was applied to a 10 ml CM Sepharose column which has been pre-equilibrated with 0.01 M sodium phosphate, pH 6. Following application to this column, the column was washed with 0.01 M sodium phosphate. Proteins were elevated from the column with a 0 - 0.5M KCl gradient in 0.01 M Na phosphate, pH 6 and fractions were collected for gel examination. Coomassie gels of column fractions were carried out to identify those fractions containing high molecular weight proteins. The fractions containing high molecular weight proteins were pooled and concentrated to a 1 to 3 ml volume in preparation for application of sample to gel filtration column.

A Sepharose CL-4B gel filtration column was equilibrated with phosphate-buffered saline, pH 7.5. The

concentrated high molecular weight protein sample was applied to the gel filtration column and column fractions were collected. Coomassie gels were performed on the column fractions to identify those containing high molecular weight proteins. The column fractions containing high molecular weight proteins were pooled.

The proteins were tested to determine whether they would protect against experimental otitis media caused by the homologous strain.

Chinchillas received three monthly subcutaneous injections with 40 μg of an HMW1-HMW2 protein mixture in Freund's adjuvant. One month after the last injection, the animals were challenged by intrabullar inoculation with 300 cfu of NTHI strain 12.

Infection developed in 5 of 5 control animals versus 5 of 10 immunized animals. Among infected animals, geometric mean bacterial counts in middle ear fluid 7 days post-challenge were 7.4×10^6 in control animals verus 1.3×10^5 in immunized animals.

Serum antibody titres following immunization were comparable in uninfected and infected animals. However, infection in immunized animals was uniformly associated with the appearance of bacteria down-regulated in expression of the HMW proteins, suggesting bacterial selection in response to immunologic pressure.

Although this data shows that protection following immunization was not complete, this data suggests the HMW adhesin proteins are potentially important protective antigens which may comprise one component of a multi-component NTHI vaccine.

Example 7:

A number of synthetic peptides were derived from HMW1. Antisera then was raised to these peptides. The anti-peptide antisera to peptide HMW1-P5 was shown to recognize HMW1. Peptide HMW1-P5 covers amino acids 1453 to 1481 of HMW1, has the sequence

VDEVIEAKRILEKVKDLSDEEREALAKLG (SEQ ID NO:9), and represents bases 1498 to 1576 in Figure 10.

This finding demonstrates that the DNA sequence and the derived protein is being interpreted in the correct reading frame and that peptides derived from the sequence can be produced which will be immunogenic.

SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides high molecular weight proteins of non-typeable Haemophilus, genes coding for the same and vaccines incorporating such proteins. Modifications are possible within the scope of this invention.

Table 1. Effect of mutation of high molecular weight proteins on adherence to Chang epithelial cells by nontypable *H. influenzae*.

Strain	ADHERENCE* % inoculum	ADHERENCE* relative to wild type†
Strain 12 derivatives		
wild type	87.7 ± 5.9	100.0 ± 6.7
HMW1- mutant	6.0 ± 0.9	6.8 ± 1.0
HMW2- mutant	89.9 ± 10.8	102.5 ± 12.3
HMW1-/HMW2- mutant	2.0 ± 0.3	2.3 ± 0.3
Strain 5 derivatives		
wild type	78.7 ± 3.2	100.0 ± 4.1
HMW1-like mutant	15.7 ± 2.6	19.9 ± 3.3
HMW2-like mutant	103.7 ± 14.0	131.7 ± 17.8
double mutant	3.5 ± 0.6	4.4 ± 0.8

* Numbers represent mean (± standard error of the mean) of measurements in triplicate or quadruplicate from representative experiments.

† Adherence values for strain 12 derivatives are relative to strain 12 wild type; values for strain 5 derivatives are relative to strain 5 wild type.

Table 2. Adherence by *E. coli* DH5α and HB101 harboring *hmw1* or *hmw2* gene clusters.

Strain*	Adherence relative to *H. influenzae* strain 12†
DH5α (pT7-7)	0.7 ± 0.02
DH5α (pHMW1-14)	114.2 ± 15.9
DH5α (pHMW2-21)	14.0 ± 3.7
HB101 (pT7-7)	1.2 ± 0.5
HB101 (pHMW1-14)	93.6 ± 15.8
HB101 (pHMW2-21)	3.6 ± 0.9

---

* The plasmid pHMW1-14 contains the *hmw1* gene cluster, while pHMW2-21 contains the *hmw2* gene cluster; pT7-7 is the cloning vector used in these constructs.

† Numbers represent the mean (± standard error of the mean) of measurements made in triplicate from representative experiments.

CLAIMS

What I claim is:

1. An isolated and purified gene encoding a high molecular weight protein of a non-typeable Haemophilus strain.

2. The gene of claim 1 encoding protein HMW1, HMW2, HMW3 or HMW4 or a variant or fragment of said protein retaining the immunological ability to protect against disease caused by a non-typeable Haemophilus strain.

3. The gene of claim 2 having the DNA sequence shown in Figure 1 and encoding protein HMW1 having the derived amino acid sequence of Figure 2.

4. The gene of claim 2 having the DNA sequence shown in Figure 3 and encoding protein HMW2 having the derived amino acid sequence of Figure 4.

5. The gene claimed in claim 2 having the partial DNA sequence shown in Figure 8 and encoding protein HMW3 having the derived amino acid sequence of Figure 10.

6. The gene claimed in claim 2 having the partial DNA sequence shown in Figure 9 and encoding protein HMW4 having the derived amino acid sequence of Figure 10.

7. A purified and isolated gene cluster comprising a nucleotide sequence for a structural gene encoding a high molecular weight protein of a non-typeable Haemophilus strain and at least one downstream nucleotide sequence for an accessory gene for effecting expression of a gene product fully encoded by said structural gene.

8. The gene cluster claimed in claim 7 comprising a DNA sequence coding for protein HMW1 or HMW2 and two downstream accessory genes.

9. The gene cluster of claim 8 having the DNA sequence shown in Figure 6.

10. The gene cluster of claim 8 having the DNA sequence shown in Figure 7.

11. A high molecular weight protein of non-typeable Haemophilus which is encoded by a gene as defined in

claim 1, or any variant or fragment thereof retaining the immunological ability to protect against disease caused by a non-typeable Haemophilus strain.

12. The protein of claim 11 which is HMW1 encoded by the DNA sequence shown in Figure 1, having the derived amino acid sequence of Figure 2 and having an apparent molecular weight of 125 kDa.

13. The protein claim 11 which is HMW2 encoded by the DNA sequence shown in Figure 3 and having the derived amino acid sequence of Figure 4 and having an apparent molecular weight of 120 kDa.

14. An isolated and purified high molecular weight protein of non-typeable Haemophilus influenzae which is antigenically related to the filamentous hemagglutinin surface protein of Bordetella pertussis.

15. The protein of claim 14 which is HMW1, HMW2, HMW3 or HMW4.

16. A conjugate comprising a protein as claimed in claim 11 or 14 linked to a antigen, hapten or polysaccharide for eliciting an immune response to said antigen, hapten or polysaccharide.

17. The conjugate as claimed in claim 16 wherein said polysaccharide is a protective polysaccharide against Haemophilus influenzae type b.

18. A synthetic peptide having an amino acid sequence corresponding to at least one protective epitope of a high molecular weight protein of non-typeable Haemophilus influenzae.

19. The peptide of claim 18 wherein said protein is HMW1, HMW2, HMW3 or HMW4.

FIG.1A. DNA SEQUENCE OF HIGH MOLECULAR WEIGHT PROTEIN I (HMW1)

```
  1  ACAGCGTTCT CTTAATACTA GTACAAACCC ACAATAAAAT ATGACAAACA
 51  ACAATTACAA CACCTTTTTT GCAGTCTATA TGCAAATATT TTAAAAAATA
101  GTATAAATCC GCCATATAAA ATGGTATAAT CTTTCATCTT TCATCTTTCA
151  TCTTTCATCT TTCATCTTTC ATCTTTCATC TTTCATCTTT CATCTTTCAT
201  CTTTCATCTT TCATCTTTCA TCTTTCATCT TTCATCTTTC ACATGCCCTG
251  ATGAACCGAG GGAAGGGAGG GAGGGGCAAG AATGAAGAGG GAGCTGAACG
301  AACGCAAATG ATAAAGTAAT TTAATTGTTC AACTAACCTT AGGAGAAAAT
351  ATGAACAAGC TATATCGTCT CAAATTCAGC AAACGCCTGA ATGCTTTGGT
401  TGCTGTGTCT GAATTGGCAC GGGGTTGTGA CCATTCCACA GAAAAAGGCA
451  GCGAAAAACC TGCTCGCATG AAAGTGCGTC ACTTAGCGTT AAAGCCACTT
501  TCCGCTATGT TACTATCTTT AGGTGTAACA TCTATTCCAC AATCTGTTTT
551  AGCAAGCGGC TTACAAGGAA TGGATGTAGT ACACGGCACA GCCACTATGC
601  AAGTAGATGG TAATAAAACC ATTATCCGCA ACAGTGTTGA CGATATCATT
651  AATTGGAAAC AATTTAACAT CGACCAAAAT GAAATGGTGC AGTTTTTACA
701  AGAAAACAAC AACTCCGCCG TATTCAACCG TGTTACATCT AACCAAATCT
```

FIG.1B.

```
 751  CCCAATTAAA AGGGATTTTA GATTCTAACG GACAAGTCTT TTTAATCAAC
 801  CCAAATGGTA TCACAATAGG TAAAGACGCA ATTATTAACA CTAATGGCTT
 851  TACGGCTTCT ACGCTAGACA TTTCTAACGA AAACATCAAG GCGCGTAATT
 901  TCACCTTCGA GCAAACCAAA GATAAAGCGC TCGCTGAAAT TGTGAATCAC
 951  GGTTTAATTA CTGTCGGTAA AGACGGCAGT GTAAATCTTA TTGGTGGCAA
1001  AGTGAAAAAC GAGGGTGTGA TTAGCGTAAA TGGTGGCAGC ATTTCTTTAC
1051  TCGCAGGGCA AAAAATCACC ATCAGCGATA TAATAAACCC AACCATTACT
1101  TACAGCATTG CCGCGCCTGA AAATGAAGCG GTCAATCTGG GCGATATTTT
1151  TGCCAAAGGC GGTAACATTA ATGTCCGTGC TGCCACTATT CGAAACCAAG
1201  GTAAACTTTC TGCTGATTCT GTAAGCAAAG ATAAAAGCGG CAATATTGTT
1251  CTTTCCGCCA AAGAGGGTGA AGCGGAAATT GGCGGTGTAA TTTCCGCTCA
1301  AAATCAGCAA GCTAAAGGCG GCAAGCTGAT GATTACAGGC GATAAAGTCA
1351  CATTAAAAAC AGGTGCAGTT ATCGACCTTT CAGGTAAAGA AGGGGGAGAA
1401  ACTTACCTTG GCGGTGACGA GCGCGGCGAA GGTAAAAAGG GCATTCAATT
1451  AGCAAAGAAA ACCTCTTTAG AAAAAGGCTC AACCATCAAT GTATCAGGCA
1501  AAGAAAAAGG CGGACGCGCT ATTGTGTGGG GCGATATTGC GTTAATTGAC
```

FIG.1C.

```
1551  GGCAATATTA ACGCTCAAGG TAGTGGTGAT ATCGCTAAAA CCGGTGGTTT
1601  TGTGGAGACG TCGGGGCATG ATTTATTCAT CAAAGACAAT GCAATTGTTG
1651  ACGCCAAAGA GTGGTTGTTA GACCCGGATA ATGTATCTAT TAATGCAGAA
1701  ACAGCAGGAC GCAGCAATAC TTCAGAAGAC GATGAATACA CGGGATCCGG
1751  GAATAGTGCC AGCACCCCAA AACGAAACAA AGAAAAGACA ACATTAACAA
1801  ACACAACTCT TGAGAGTATA CTAAAAAAAG GTACCTTTGT TAACATCACT
1851  GCTAATCAAC GCATCTATGT CAATAGCTCC ATTAATTTAT CCAATGGCAG
1901  CTTAACTCTT TGGAGTGAGG GTCGGAGCGG TGGCGGCGTT GAGATTAACA
1951  ACGATATTAC CACCGGTGAT GATACCAGAG GTGCAAACTT AACAATTTAC
2001  TCAGGCGGCT GGGTTGATGT TCATAAAAAT ATCTCACTCG GGGCGCAAGG
2051  TAACATAAAC ATTACAGCTA AACAAGATAT CGCCTTTGAG AAAGGAAGCA
2101  ACCAAGTCAT TACAGGTCAA GGGACTATTA CCTCAGGCAA TCAAAAAGGT
2151  TTTAGATTTA ATAATGTCTC TCTAAACGGC ACTGGCAGCG GACTGCAATT
2201  CACCACTAAA AGAACCAATA AATACGCTAT CACAAATAAA TTTGAAGGGA
2251  CTTTAAATAT TTCAGGGAAA GTGAACATCT CAATGGTTTT ACCTAAAAAT
2301  GAAAGTGGAT ATGATAAATT CAAAGGACGC ACTTACTGGA ATTTAACCTC
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FIG.1D.

```
2351  CTTAAATGTT TCCGAGAGTG GCGAGTTTAA CCTCACTATT GACTCCAGAG
2401  GAAGCGATAG TGCAGGCACA CTTACCCAGC CTTATAATTT AAACGGTATA
2451  TCATTCAACA AAGACACTAC CTTTAATGTT GAACGAAATG CAAGAGTCAA
2501  CTTTGACATC AAGGCACCAA TAGGGATAAA TAAGTATTCT AGTTTGAATT
2551  ACGCATCATT TAATGGAAAC ATTTCAGTTT CGGGAGGGGG GAGTGTTGAT
2601  TTCACACTTC TCGCCTCATC CTCTAACGTC CAAACCCCCG GTGTAGTTAT
2651  AAATTCTAAA TACTTTAATG TTTCAACAGG GTCAAGTTTA AGATTTAAAA
2701  CTTCAGGCTC AACAAAAACT GGCTTCTCAA TAGAGAAAGA TTTAACTTTA
2751  AATGCCACCG GAGGCAACAT AACACTTTTG CAAGTTGAAG GCACCGATGG
2801  AATGATTGGT AAAGGCATTG TAGCCAAAAA AAACATAACC TTTGAAGGAG
2851  GTAACATCAC CTTTGGCTCC AGGAAAGCCG TAACAGAAAT CGAAGGCAAT
2901  GTTACTATCA ATAACAACGC TAACGTCACT CTTATCGGTT CGGATTTTGA
2951  CAACCATCAA AAACCTTTAA CTATTAAAAA AGATGTCATC ATTAATAGCG
3001  GCAACCTTAC CGCTGGAGGC AATATTGTCA ATATAGCCGG AAATCTTACC
3051  GTTGAAAGTA ACGCTAATTT CAAAGCTATC ACAAATTTCA CTTTTAATGT
3101  AGGCGGCTTG TTTGACAACA AAGGCAATTC AAATATTTCC ATTGCCAAAG
3151  GAGGGGCTCG CTTTAAAGAC ATTGATAATT CCAAGAATTT AAGCATCACC
```

FIG.1E.

```
3201  ACCAACTCCA GCTCCACTTA CCGCACTATT ATAAGCGGCA ATATAACCAA
3251  TAAAAACGGT GATTTAAATA TTACGAACGA AGGTAGTGAT ACTGAAATGC
3301  AAATTGGCGG CGATGTCTCG CAAAAAGAAG GTAATCTCAC GATTTCTTCT
3351  GACAAAATCA ATATTACCAA ACAGATAACA ATCAAGGCAG GTGTTGATGG
3401  GGAGAATTCC GATTCAGACG CGACAAACAA TGCCAATCTA ACCATTAAAA
3451  CCAAAGAATT GAAATTAACG CAAGACCTAA ATATTTCAGG TTTCAATAAA
3501  GCAGAGATTA CAGCTAAAGA TGGTAGTGAT TTAACTATTG GTAACACCAA
3551  TAGTGCTGAT GGTACTAATG CCAAAAAAGT AACCTTTAAC CAGGTTAAAG
3601  ATTCAAAAAT CTCTGCTGAC GGTCACAAGG TGACACTACA CAGCAAAGTG
3651  GAAACATCCG GTAGTAATAA CAACACTGAA GATAGCAGTG ACAATAATGC
3701  CGGCTTAACT ATCGATGCAA AAAATGTAAC AGTAAACAAC AATATTACTT
3751  CTCACAAAGC AGTGAGCATC TCTGCGACAA GTGGAGAAAT TACCACTAAA
3801  ACAGGTACAA CCATTAACGC AACCACTGGT AACGTGGAGA TAACCGCTCA
3851  AACAGGTAGT ATCCTAGGTG GAATTGAGTC CAGCTCTGGC TCTGTAACAC
3901  TTACTGCAAC CGAGGGCGCT CTTGCTGTAA GCAATATTTC GGGCAACACC
3951  GTTACTGTTA CTGCAAATAG CGGTGCATTA ACCACTTTGG CAGGCTCTAC
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FIG.1F.

```
4001  AATTAAAGGA ACCGAGAGTG TAACCACTTC AAGTCAATCA GGCGATATCG
4051  GCGGTACGAT TTCTGGTGGC ACAGTAGAGG TTAAAGCAAC CGAAAGTTTA
4101  ACCACTCAAT CCAATTCAAA AATTAAAGCA ACAACAGGCG AGGCTAACGT
4151  AACAAGTGCA ACAGGTACAA TTGGTGGTAC GATTTCCGGT AATACGGTAA
4201  ATGTTACGGC AAACGCTGGC GATTTAACAG TTGGGAATGG CGCAGAAATT
4251  AATGCGACAG AAGGAGCTGC AACCTTAACT ACATCATCGG GCAAATTAAC
4301  TACCGAAGCT AGTTCACACA TTACTTCAGC CAAGGGTCAG GTAAATCTTT
4351  CAGCTCAGGA TGGTAGCGTT GCAGGAAGTA TTAATGCCGC CAATGTGACA
4401  CTAAATACTA CAGGCACTTT AACTACCGTG AAGGGTTCAA ACATTAATGC
4451  AACCAGCGGT ACCTTGGTTA TTAACGCAAA AGACGCTGAG CTAAATGGCG
4501  CAGCATTGGG TAACCACACA GTGGTAAATG CAACCAACGC AAATGGCTCC
4551  GGCAGCGTAA TCGCGACAAC CTCAAGCAGA GTGAACATCA CTGGGGATTT
4601  AATCACAATA AATGGATTAA ATATCATTTC AAAAAACGGT ATAAACACCG
4651  TACTGTTAAA AGGCGTTAAA ATTGATGTGA AATACATTCA ACCGGGTATA
4701  GCAAGCGTAG ATGAAGTAAT TGAAGCGAAA CGCATCCTTG AGAAGGTAAA
4751  AGATTTATCT GATGAAGAAA GAGAAGCGTT AGCTAAACTT GGAGTAAGTG
4801  CTGTACGTTT TATTGAGCCA AATAATACAA TTACAGTCGA TACACAAAAT
```

FIG.1G.

```
4851  GAATTTGCAA CCAGACCATT AAGTCGAATA GTGATTTCTG AAGGCAGGGC
4901  GTGTTTCTCA AACAGTGATG GCGCGACGGT GTGCGTTAAT ATCGCTGATA
4951  ACGGGCGGTA GCGGTCAGTA ATTGACAAGG TAGATTTCAT CCTGCAATGA
5001  AGTCATTTTA TTTTCGTATT ATTTACTGTG TGGGTTAAAG TTCAGTACGG
5051  GCTTTACCCA TCTTGTAAAA AATTACGGAG AATACAATAA AGTATTTTTA
5101  ACAGGTTATT ATTATG
```

FIG.2A. AMINO ACID SEQUENCE OF HIGH MOLECULAR WEIGHT PROTEIN I

```
  1  MNKIYRLKFS KRLNALVAVS ELARGCDHST EKGSEKPARM KVRHLALKPL
 51  SAMLLSLGVT SIPQSVLASG LQGMDVVHGT ATMQVDGNKT IIRNSVDAII
101  NWKQFNIDQN EMVQFLQENN NSAVFNRVTS NQISQLKGIL DSNGQVFLIN
151  PNGITIGKDA IINTNGFTAS TLDISNENIK ARNFTFEQTK DKALAEIVNH
201  GLITVGKDGS VNLIGGKVKN EGVISVNGGS ISLLAGQKIT ISDIINPTIT
251  YSIAAPENEA VNLGDIFAKG GNINVRAATI RNQGKLSADS VSKDKSGNIV
301  LSAKEGEAEI GGVISAQNQQ AKGGKLMITG DKVTLKTGAV IDLSGKEGGE
351  TYLGGDERGE GKNGIQLAKK TSLEKGSTIN VSGKEKGGRA IVWGDIALID
401  GNINAQGSGD IAKTGGFVET SGHDLFIKDN AIVDAKEWLL DFDNVSINAE
451  TAGRSNTSED DEYTGSGNSA STPKRNKEKT TLTNTTLESI LKKGTFVNIT
501  ANQRIYVNSS INLSNGSLTL WSEGRSGGGV EINNDITTGD DTRGANLTIY
551  SGGWVDVHKN ISLGAQGNIN ITAKQDIAFE KGSNQVITGQ GTITSGNQKG
601  FRFNNVSLNG TGSGLQFTTK RTNKYAITNK FEGTLNISGK VNISMVLPKN
651  ESGYDKFKGR TYWNLTSLNV SESGEFNLTI DSRGSDSAGT LTQPYNLNGI
701  SFNKDTTFNV ERNARVNFDI KAPIGINKYS SLNYASFNGN ISVSGGGSVD
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PCT/US93/02166

FIG.2B.

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 751  FTLLASSSNV QTPGVVINSK YFNVSTGSSL RFKTSGSTKT GFSIEKDLTL
 801  NATGGNITLL QVEGTDGMIG KGIVAKKNIT FEGGNITFGS RKAVTEIEGN
 851  VTINNNANVT LIGSDFDNHQ KPLTIKKDVI INSGNLTAGG NIVNIAGNLT
 901  VESNANFKAI TNFTFNVGGL FDNKGNSNIS IAKGGARFKD IDNSKNLSIT
 951  TNSSSTYRTI ISGNITNKNG DLNITNEGSD TEMQIGGDVS QKEGNLTISS
1001  DKINITKQIT IKAGVDGENS DSDATNNANL TIKTKELKLT QDLNISGFNK
1051  AEITAKDGSD LTIGNTNSAD GTNAKKVTFN QVKDSKISAD GHKVTLHSKV
1101  ETSGSNNNTE DSSDNNAGLT IDAKNVTVNN NITSHKAVSI SATSGEITTK
1151  TGTTINATTG NVEITAQTGS ILGGIESSSG SVTLTATEGA LAVSNISGNT
1201  VTVTANSGAL TTLAGSTIKG TESVTTSSQS GDIGGTISGG TVEVKATESL
1251  TTQSNSKIKA TTGEANVTSA TGTIGGTISG NTVNVTANAG DLTVGNGAEI
1301  NATEGAATLT TSSGKLTTEA SSHITSAKGQ VNLSAQDGSV AGSINAANVT
1351  LNTTGTLTTV KGSNINATSG TLVINAKDAE LNGAALGNHT VVNATNANGS
1401  GSVIATTSSR VNITGDLITI NGLNIISKNG INTVLLKGVK IDVKYIQPGI
1451  ASVDEVIEAK RILEKVKDLS DEEREALAKL GVSAVRFIEP NNTITVDTQN
1501  EFATRPLSRI VISEGRACFS NSDGATVCVN IADNGR
```

FIG. 3A. AMINO ACID SEQUENCE OF HIGH MOLECULAR WEIGHT PROTEIN II (HMW2)

```
   1  TAAATATACA AGATAATAAA AATAAATCAA GATTTTTGTG ATGACAAACA
  51  ACAATTACAA CACCTTTTTT GCAGTCTATA TGCAAATATT TTAAAAAAAT
 101  AGTATAAATC CGCCATATAA AATGGTATAA TCTTTCATCT TTCATCTTTA
 151  ATCTTTCATC TTTCATCTTT CATCTTTCAT CTTTCATCTT TCATCTTTCA
 201  TCTTTCATCT TTCATCTTTC ATCTTTCATC TTTCATCTTT CACATGAAAT
 251  GATGAACCGA GGGAAGGGAG GGAGGGGCAA GAATGAAGAG GGAGCTGAAC
 301  GAACGCAAAT GATAAAGTAA TTTAATTGTT CAACTAACCT TAGGAGAAAA
 351  TATGAACAAG ATATATCGTC TCAAATTCAG CAAACGCCTG AATGCTTTGG
 401  TTGCTGTGTC TGAATTGGCA CGGGGTTGTG ACCATTCCAC AGAAAAAGGC
 451  TTCCGCTATG TTACTATCTT TAGGTGTAAC CACTTAGCGT TAAAGCCACT
 501  TTCCGCTATG TTACTATCTT TAGGTGTAAC ATCTATTCCA CAATCTGTTT
 551  TAGCAAGCGG CTTACAAGGA ATGGATGTAG TACACGGCAC AGCCACTATG
 601  CAAGTAGATG GTAATAAAAC CATTATCCGC AACAGTGTTG ACGCTATCAT
 651  TAATTGGAAA CAATTTAACA TCGACCAAAA TGAAATGGTG CAGTTTTTAC
 701  AAGAAAACAA CAACTCCGCC GTATTCAACC GTGTTACATC TAACCAAATC
```

FIG.3B.

```
 751  TCCCAATTAA AAGGGATTTT AGATTCTAAC GGACAAGTCT TTTTAATCAA
 801  CCCAAATGGT ATCACAATAG GTAAAGACGC AATTATTAAC ACTAATGGCT
 851  TTACGGCTTC TACGCTAGAC ATTTCTAACG AAAACATCAA GGCGCGTAAT
 901  TTCACCTTCG AGCAAACCAA AGATAAAGCG CTCGCTGAAA TTGTGAATCA
 951  CGGTTTAATT ACTGTCGGTA AAGACGGCAG TGTAAATCTT ATTGGTGGCA
1001  AAGTGAAAAA CGAGGGTGTG ATTAGCGTAA ATGGTGGCAG CATTTCTTTA
1051  CTCGCAGGGC AAAAAATCAC CATCAGCGAT ATAATAAACC CAACCATTAC
1101  TTACAGCATT GCCGCGCCTG AAAATGAAGC GGTCAATCTG GGCGATATTT
1151  TTGCCAAAGG CGGTAACATT AATGTCCGTG CTGCCACTAT TCGAAACCAA
1201  GGTAAACTTT CTGCTGATTC TGTAAGCAAA GATAAAAGCG GCAATATTGT
1251  TCTTTCCGCC AAAGAGGGTG AAGCGGAAAT TGGCGGTGTA ATTTCCGCTC
1301  AAAATCAGCA AGCTAAAGGC GGCAAGCTGA TGATTACAGG CGATAAAGTC
1351  ACATTAAAAA CAGGTGCAGT TATCGACCTT TCAGGTAAAG AAGGGGGAGA
1401  AACTTACCTT GGCGGTGACG AGCGCGGCGA AGGTAAAAAC GGCATTCAAT
1451  TAGCAAAGAA AACCTCTTTA GAAAAAGGCT CAACCATCAA TGTATCAGGC
1501  AAAGAAAAAG GCGGACGCGC TATTGTGTGG GGCGATATTG CGTTAATTGA
```

FIG.3C.

```
1551  CGGCAATATT AACGCTCAAG GTAGTGGTGA TATCGCTAAA ACCGGTGGTT
1601  TTGTGGAGAC ATCGGGGCAT TATTTATCCA TTGACAGCAA TGCAATTGTT
1651  AAAACAAAAG AGTGGTTGCT AGACCCTGAT GATGTAACAA TTGAAGCCGA
1701  AGACCCCCTT CGCAATAATA CCGGTATAAA TGATGAATTC CCAACAGGCA
1751  CCGGTGAAGC AAGCGACCCT AAAAAAAATA GCGAACTCAA AACAACGCTA
1801  ACCAATACAA CTATTTCAAATTATCTGAAA AACGCCTGGA CAATGAATAT
1851  AACGGCATCA AGAAAACTTA CCGTTAATAG CTCAATCAAC ATCGGAAGCA
1901  ACTCCCACTT AATTCTCCAT AGTAAAGGTC AGCGTGGCGG AGGCGTTCAG
1951  ATTGATGGAG ATATTACTTC TAAAGGCGGA AATTTAACCA TTTATTCTGG
2001  CGGATGGGTT GATGTTCATA AAAATATTAC GCTTGATCAG GGTTTTTTAA
2051  ATATTACCGC CGCTTCCGTA GCTTTTGAAG GTGGAAATAA CAAAGCACGC
2101  GACGCGGCAA ATGCTAAAAT TGTCGCCCAG GGCACTGTAA CCATTACAGG
2151  AGAGGGAAAA GATTTCAGGG CTAACAACGT ATCTTTAAAC GGAACGGGTA
2201  AAGGTCTGAA TATCATTTCA TCAGTGAATA ATTTAACCCA CAATCTTAGT
2251  GGCACAATTA ACATATCTGG GAATATAACA ATTAACCAAA CTACGAGAAA
2301  GAACACCTCG TATTGGCAAA CCAGCCATGA TTCGCACTGG AACGTCAGTG
2351  CTCTTAATCT AGAGACAGGC GCAAATTTTA CCTTTATTAA ATACATTTCA
```

FIG.3D.

```
2401  AGCAATAGCA AAGGCTTAAC AACACAGTAT AGAAGCTCTG CAGGGGTGAA
2451  TTTTAACGGC GTAAATGGCA ACATGTCATT CAATCTCAAA GAAGGAGCGA
2501  AAGTTAATTT CAAATTAAAA CCAAACGAGA ACATGAACAC AAGCAAACCT
2551  TTACCAATTC GGTTTTTAGC CAATATCACA GCCACTGGTG GGGGCTCTGT
2601  TTTTTTTGAT ATATATGCCA ACCATTCTGG CAGAGGGGCT GAGTTAAAAA
2651  TGAGTGAAAT TAATATCTCT AACGGCGCTA ATTTTACCTT AAATTCCCAT
2701  GTTCGCGGCG ATGACGCTTT TAAAATCAAC AAAGACTTAA CCATAAATGC
2751  AACCAATTCA AATTTCAGCC TCAGACAGAC GAAAGATGAT TTTTATGACG
2801  GGTACGCACG CAATGCCATC AATTCAACCT ACAACATATC CATTCTGGGC
2851  GGTAATGTCA CCCTTGGTGG ACAAAACTCA AGCAGCAGCA TTACGGGGAA
2901  TATTACTATC GAGAAAGCAG CAAATGTTAC GCTAGAAGCC AATAACGCCC
2951  CTAATCAGCA AAACATAAGG GATAGAGTTA TAAAACTTGG CAGCTTGCTC
3001  GTTAATGGGA GTTTAAGTTT AACTGGCGAA AATGCAGATA TTAAAGGCAA
3051  TCTCACTATT TCAGAAAGCG CCACTTTTAA AGGAAAGACT AGAGATACCC
3101  TAAATATCAC CGGCAATTTT ACCAATAATG GCACTGCCGA AATTAATATA
3151  ACACAAGGAG TGGTAAAACT TGGCAATGTT ACCAATGATG GTGATTTAAA
```

FIG.3E.

```
3201  CATTACCACT CACGCTAAAC GCAACCAAAG AAGCATCATC GGCGGAGATA
3251  TAATCAACAA AAAAGGAAGC TTAAATATTA CAGACAGTAA TAATGATGCT
3301  GAAATCCAAA TTGGCGGCAA TATCTCGCAA AAAGAAGGCA ACCTCACGAT
3351  TTCTTCCGAT AAAATTAATA TCACCAAACA GATAACAATC AAAAAGGGTA
3401  TTGATGGAGA GGACTCTAGT TCAGATGCGA CAAGTAATGC CAACCTAACT
3451  ATTAAAACCA AAGAATTGAA ATTGACAGAA GACCTAAGTA TTTCAGGTTT
3501  CAATAAAGCA GAGATTACAG CCAAAGATGG TAGAGATTTA ACTATTGGCA
3551  ACAGTAATGA CGGTAACAGC GGTGCCGAAG CCAAAACAGT AACTTTTAAC
3601  AATGTTAAAG ATTCAAAAAT CTCTGCTGAC GGTCACAATG TGACACTAAA
3651  TAGCAAAGTG AAAACATCTA GCAGCAATGG CGGACGTGAA AGCAATAGCG
3701  ACAACGATAC CGGCTTAACT ATTACTGCAA AAAATGTAGA AGTAAACAAA
3751  GATATTACTT CTCTCAAAAC AGTAAATATC ACCGCGTCGG AAAAGGTTAC
3801  CACCACAGCA GGCTCGACCA TTAACGCAAC AAATGGCAAA GCAAGTATTA
3851  CAACCAAAAC AGGTGATATC AGCGGTACGA TTTCCGGTAA CACGGTAAGT
3901  GTTAGCGCGA CTGGTGATTT AACCACTAAA TCCGGCTCAA AAATTGAAGC
3951  GAAATCGGGT GAGGCTAATG TAACAAGTGC AACAGGTACA ATTGGCGGTA
```

FIG.3F.

```
4001  CAATTTCCGG TAATACGGTA AATGTTACGG CAAACGCTGG CGATTTAACA
4051  GTTGGGAATG GCGCAGAAAT TAATGCGACA GAAGGAGCTG CAACCTTAAC
4101  CGCAACAGGG AATACCTTGA CTACTGAAGC CGGTTCTAGC ATCACTTCAA
4151  CTAAGGGTCA GGTAGACCTC TTGGCTCAGA ATGGTAGCAT CGCAGGAAGC
4201  ATTAATGCTG CTAATGTGAC ATTAAATACT ACAGGCACCT TAACCACCGT
4251  GGCAGGCTCG GATATTAAAG CAACCAGCGG CACCTTGGTT ATTAACGCAA
4301  AAGATGCTAA GCTAAATGGT GATGCATCAG GTGATAGTAC AGAAGTGAAT
4351  GCAGTCAACG CAAGCGGCTC TGGTAGTGTG ACTGCGGCAA CCTCAAGCAG
4401  TGTGAATATC ACTGGGGATT TAAACACAGT AAATGGGTTA AATATCATTT
4451  CGAAAGATGG TAGAAACACT GTGCGCTTAA GAGGCAAGGA AATTGAGGTG
4501  AAATATATCC AGCCAGGTGT AGCAAGTGTA GAAGAAGTAA TTGAAGCGAA
4551  ACGCGTCCTT GAAAAAGTAA AAGATTTATC TGATGAAGAA AGAGAAACAT
4601  TAGCTAAACT TGGTGTAAGT GCTGTACGTT TTGTTGAGCC AAATAATACA
4651  ATTACAGTCA ATACACAAAA TGAATTTACA ACCAGACCGT CAAGTCAAGT
4701  GATAATTTCT GAAGGTAAGG CGTGTTTCTC AAGTGGTAAT GGCGCACGAG
4751  TATGTACCAA TGTTGCTGAC GATGGACAGC CGTAGTCAGT AATTGACAAG
4801  GTAGATTTCA TCCTGCAATG AAGTCATTTT ATTTTCGTAT TATTTACTGT
```

FIG.3G.

```
4851  GTGGGTTAAA GTTCAGTACG GGCTTTACCC ATCTTGTAAA AAATTACGGA
4901  GAATACAATA AAGTATTTTT AACAGGTTAT TATTATG
```

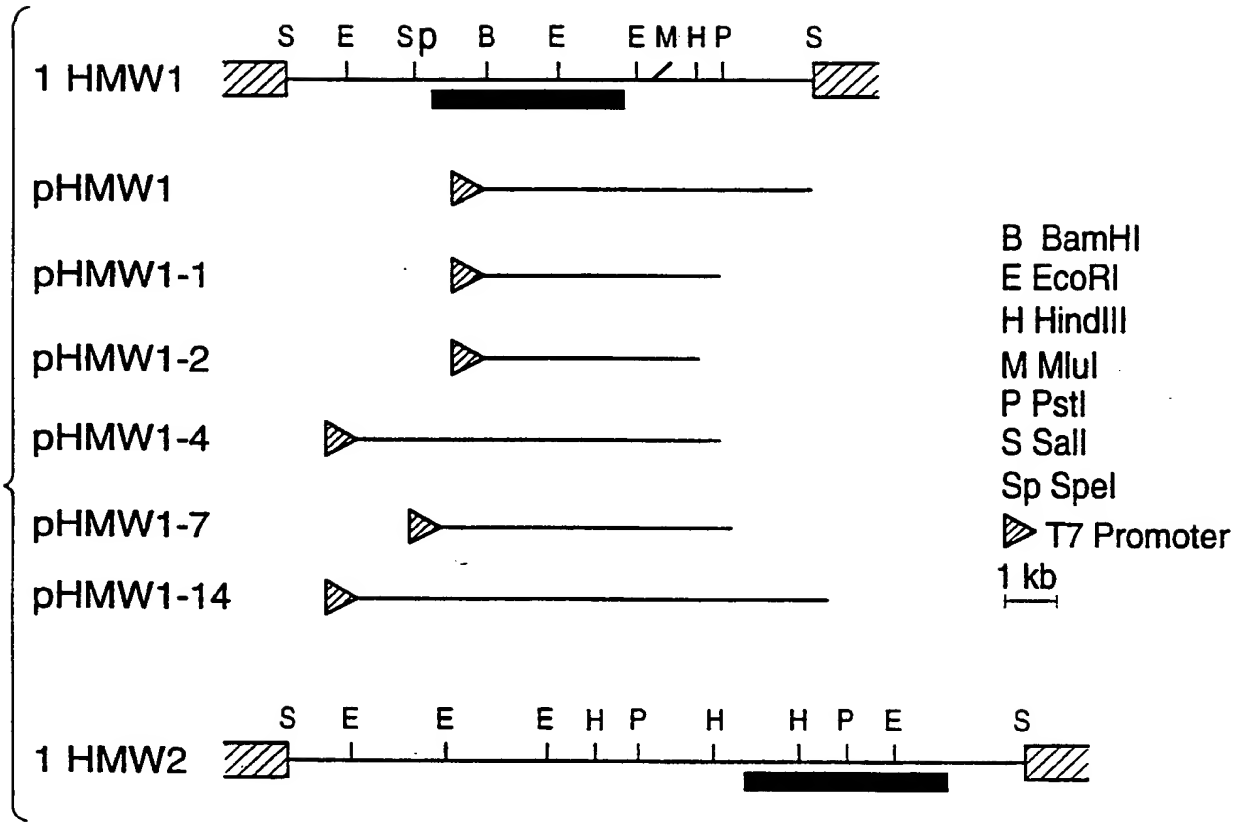
FIG.4A. AMINO ACID SEQUENCE OF HIGH MOLECULAR WEIGHT PROTEIN 2

```
  1  MNKIYRLKFS KRLNALVAVS ELARGCDHST EKGSEKPARM KVRHLALKPL
 51  SAMLLSLGVT SIPQSVLASG LQGMDVVHGT ATMQVDGNKT IIRNSVDAII
101  NWKQFNIDQN EMVQFLQENN NSAVFNRVTS NQISQLKGIL DSNGQVFLIN
151  PNGITIGKDA IINTNGFTAS TLDISNENIK ARNFTFEQTK DKALAEIVNH
201  GLITVGKDGS VNLIGGKVKN EGVISVNGGS ISLLAGQKIT ISDIINPTIT
251  YSIAAPENEA VNLGDIFAKG GNINVRAATI RNQGKLSADS VSKDKSGNIV
301  LSAKEGEAEI GGVISAQNQQ AKGGKLMITG DKVTLKTGAV IDLSGKEGGE
351  TYLGGDERGE GKNGIQLAKK TSLEKGSTIN VSGKEKGGRA IVWGDIALID
401  GNINAQGSGD IAKTGGFVET SGHDLFIKDN AIVDAKEWLL DFDNVSINAE
451  DPLRNNTGIN DEFPTGTGEA SDPKKNSELK TTLTNTTISN YLKNAWTMNI
501  TASRKLTVNS SINIGSNSHL ILHSKGQRGG GVQIDGDITS KGGNLTIYSG
551  GWVDVHKNIT LDQGFLNITA ASVAFEGGNN KARDAANAKI VAQGTVTITG
601  EGKDFRANNV SLNGTGKGLN IISSVNNLTH NLSGTINISG NITINQTTRK
651  NTSYWQTSHD SHWNVSALNL ETGANFTFIK YISSNSKGLT TQYRSSAGVN
701  FNGVNGNMSF NLKEGAKVNF KLKPNENMNT SKPLPIRFLA NITATGGGSV
```


PCT/US93/02166

FIG.4B.

```
 751  FFDIYANHSG RGAELKMSEI NISNGANFTL NSHVRGDDAF KINKDLTINA
 801  TNSNFSLRQT KDDFYDGYAR NAINSTYNIS ILGGNVTLGG QNSSSSITGN
 851  ITIEKAANVT LEANNAPNQQ NIRDRVIKLG SLLVNGSLSL TGENADIKGN
 901  LTISESATFK GKTRDTLNIT GNFTNNGTAE INITQGVVKL GNVTNDGDLN
 951  ITTHAKRNQR SIIGGDIINK KGSLNITDSN NDAEIQIGGN ISQKEGNLTI
1001  SSDKINITKQ ITIKKGIDGE DSSSDATSNA NLTIKTKELK LTEDLSISGF
1051  NKAEITAKDG RDLTIGNSND GNSGAEAKTV TFNNVKDSKI SADGHNVTLN
1101  SKVKTSSSNG GRESNSDNDT GLTITAKNVE VNKDITSLKT VNITASEKVT
1151  TTAGSTINAT NGKASITTKT GDISGTISGN TVSVSATVDL TTKSGSKIEA
1201  KSGEANVTSA TGTIGGTISG NTVNVTANAG DLTVGNGAEI NATEGAATLT
1251  ATGNTLTTEA GSSITSTKGQ VDLLAQNGSI AGSINAANVT LNTTGTLTTV
1301  AGSDIKATSG TLVINAKDAK LNGDASGDST EVNAVNASGS GSVTAATSSS
1351  VNITGDLNTV NGLNIISKDG RNTVRLRGKE IEVKYIQPGV ASVEEVIEAK
1401  RVLEKVKDLS DEERETLAKL GVSAVRFVEP NNTITVNTQN EFTTRPSSQV
1451  IISEGKACFS SGNGARVCTN VADDGQP
```


S E Sp B E E M H P S
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pHMW1
pHMW1-1
pHMW1-2
pHMW1-4
pHMW1-7
pHMW1-14
S E E E H P H H P E S
1 HMW2
B BamHI
E EcoRI
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M MluI
P PstI
S SalI
Sp SpeI
T7 Promoter
1 kb

FIG.5A.

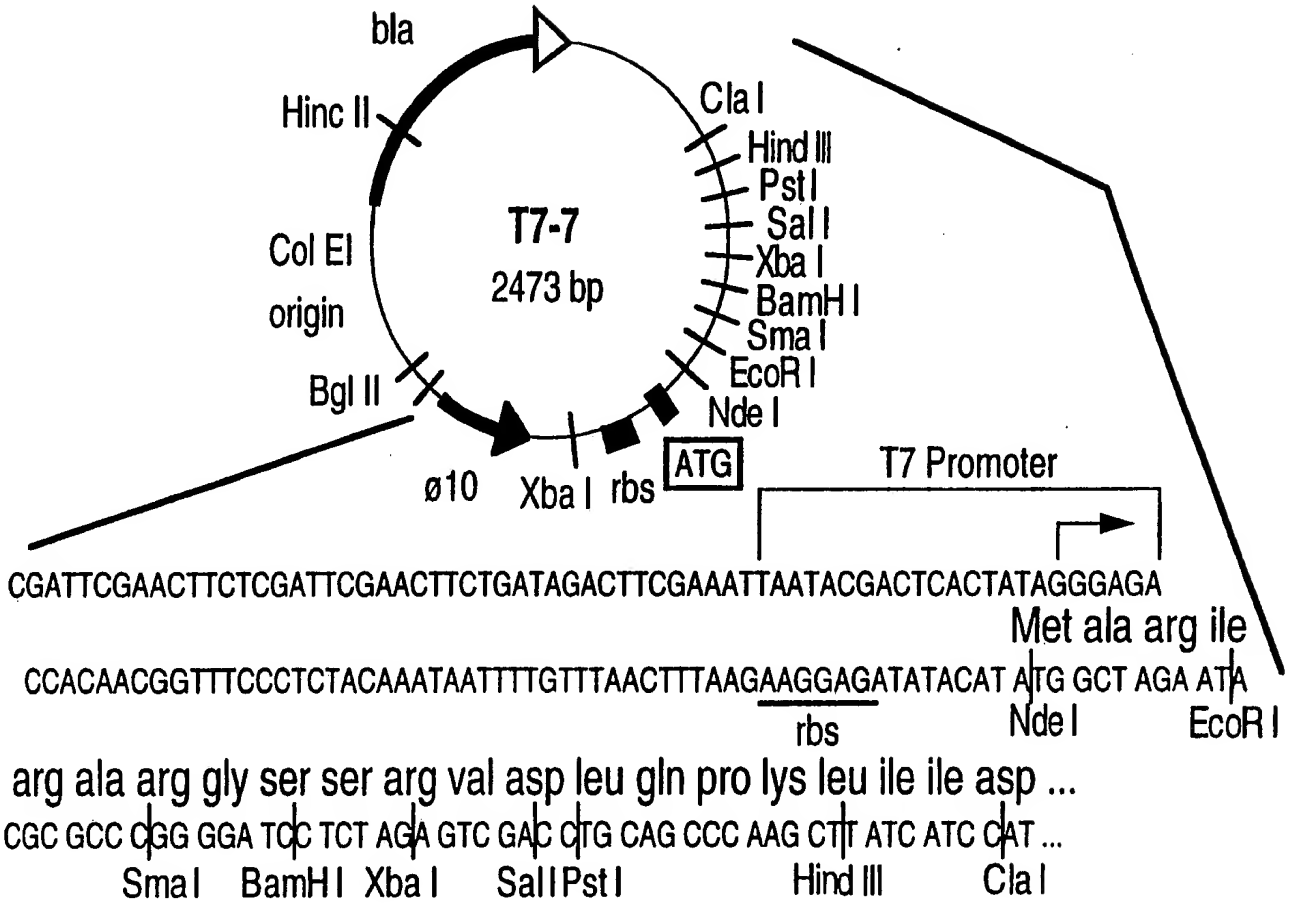

FIG.5B.

(A) Partial restriction maps of representative HMW1 and HMW2 recombinant phage and of HMW1 plasmid subclones. The shaded boxes indicate the locations of the structural genes. In the recombinant phage, transcription proceeds from left to right for the HMW1 gene and from right to left for the HMW2 gene. The methods used for construction of the plasmids shown are described in the text. (B) Restriction map of the T7 expression vector pT7-7. This vector contains the T7 RNA polymerase promoter ϕ10, a ribosome - binding site (rbs), and the translational start site for the T7 gene 10 protein upstream from a multiple cloning site (37).

FIG.6A.

```
  1  ACAGCGTTCT CTTAATACTA GTACAAACCC ACAATAAAAT ATGACAAACA
 51  ACAATTACAA CACCTTTTTT GCAGTCTATA TGCAAATATT TTAAAAAATA
101  GTATAAATCC GCCATATAAA ATGGTATAAT CTTTCATCTT TCATCTTTCA
151  TCTTTCATCT TTCATCTTTC ATCTTTCATC TTTCATCTTT CATCTTTCAT
201  CTTTCATCTT TCATCTTTCA TCTTTCATCT TTCATCTTTC ACATGAAATG
251  ATGAACCGAG GGAAGGGAGG GAGGGGCAAG AATGAAGAGG GAGCTGAACG
301  AACGCAAATG ATAAAGTAAT TTAATTGTTC AACTAACCTT AGGAGAAAAT
351  ATGAACAAGA TATATCGTCT CAAATTCAGC AAACGCCTGA ATGCTTTGGT
401  TGCTGTGTCT GAATTGGCAC GGGGTTGTGA CCATTCCACA GAAAAAGGCA
451  GCGAAAAACC TGCTCGCATG AAAGTGCGTC ACTTAGCGTT AAAGCCACTT
501  TCCGCTATGT TACTATCTTT AGGTGTAACA TCTATTCCAC AATCTGTTTT
551  AGCAAGCGGC TTACAAGGAA TGGATGTAGT ACACGGCACA GCCACTATGC
601  AAGTAGATGG TAATAAAACC ATTATCCGCA ACAGTGTTGA CGCTATCATT
651  AATTGGAAAC AATTTAACAT CGACCAAAAT GAAATGGTGC AGTTTTTACA
701  AGAAAACAAC AACTCCGCCG TATTCAACCG TGTTACATCT AACCAAATCT
751  CCCAATTAAA AGGGATTTTA GATTCTAACG GACAAGTCTT TTTAATCAAC
```

FIG.6B.

```
 801  CCAAATGGTA TCACAATAGG TAAAGACGCA ATTATTAACA CTAATGGCTT
 851  TACGGCTTCT ACGCTAGACA TTTCTAACGA AAACATCAAG GCGCGTAATT
 901  TCACCTTCGA GCAAACCAAA GATAAAGCGC TCGCTGAAAT TGTGAATCAC
 951  GGTTTAATTA CTGTCGGTAA AGACGGCAGT GTAAATCTTA TTGGTGGCAA
1001  AGTGAAAAAC GAGGGTGTGA TTAGCGTAAA TGGTGGCAGC ATTTCTTTAC
1051  TCGCAGGGCA AAAAATCACC ATCAGCGATA TAATAAACCC AACCATTACT
1101  TACAGCATTG CCGCGCCTGA AAATGAAGCG GTCAATCTGG GCGATATTTT
1151  TGCCAAAGGC GGTAACATTA ATGTCCGTGC TGCCACTATT CGAAACCAAG
1251  CTTTCCGCCA AAGAGGGTGA AGCGGAAATT GGCGGTGTAA TTTCCGCTCA
1301  AAATCAGCAA GCTAAAGGCG GCAAGCTGAT GATTACAGGC GATAAAGTCA
1351  CATTAAAAAC AGGTGCAGTT ATCGACCTTT CAGGTAAAGA AGGGGGAGAA
1401  ACTTACCTTG GCGGTGACGA GCGCGGCGAA GGTAAAAACG GCATTCAATT
1451  AGCAAAGAAA ACCTCTTTAG AAAAAGGCTC AACCATCAAT GTATCAGGCA
1501  AAGAAAAAGG CGGACGCGCT ATTGTGTGGG GCGATATTGC GTTAATTGAC
1551  GGCAATATTA ACGCTCAAGG TAGTGGTGAT ATCGCTAAAA CCGGTGGTTT
1601  TGTGGAGACG TCGGGGCATG ATTTATTCAT CAAAGACAAT GCAATTGTTG
```

FIG.6C.

```
1651  ACGCCAAAGA GTGGTTGTTA GACCCGGATA ATGTATCTAT TAATGCAGAA
1701  ACAGCAGGAC GCAGCAATAC TTCAGAAGAC GATGAATACA CGGGATCCGG
1751  GAATAGTGCC AGCACCCCAA AACGAAACAA AGAAAAGACA ACATTAACAA
1801  ACACAACTCT TGAGAGTATA CTAAAAAAAG GTACCTTTGT TAACATCACT
1851  GCTAATCAAC GCATCTATGT CAATAGCTCC ATTAATTTAT CCAATGGCAG
1901  CTTAACTCTT TGGAGTGAGG GTCGGAGCGG TGGCGGCGTT GAGATTAACA
1951  ACGATATTAC CACCGGTGAT GATACCAGAG GTGCAAACTT AACAATTTAC
2001  TCAGGCGGCT GGGTTGATGT TCATAAAAAT ATCTCACTCG GGGCGCAAGG
2051  TAACATAAAC ATTACAGCTA AACAAGATAT CGCCTTTGAG AAAGGAAGCA
2101  ACCAAGTCAT TACAGGTCAA GGGACTATTA CCTCAGGCAA TCAAAAAGGT
2151  TTTAGATTTA ATAATGTCTC TCTAAACGGC ACTGGCAGCG GACTGCAATT
2201  CACCACTAAA AGAACCAATA AATACGCTAT CACAAATAAA TTTGAAGGGA
2251  CTTTAAATAT TTCAGGGAAA GTGAACATCT CAATGGTTTT ACCTAAAAAT
2301  GAAAGTGGAT ATGATAAATT CAAAGGACGC ACTTACTGGA ATTTAACCTC
2351  GAAAGTGGAT ATGATAAATT CAAAGGACGC CCTCACTATT GACTCCAGAG
2401  GAAGCGATAG TGCAGGCACA CTTACCCAGC CTTATAATTT AAACGGTATA
2451  TCATTCAACA AAGACACTAC CTTTAATGTT GAACGAAATG CAAGAGTCAA
```

FIG.6D.

```
2501  CTTTGACATC AAGGCACCAA TAGGGATAAA TAAGTATTCT AGTTTGAATT
2551  ACGCATCATT TAATGGAAAC ATTTCAGTTT CGGGAGGGGG GAGTGTTGAT
2601  TTCACACTTC TCGCCTCATC CTCTAACGTC CAAACCCCCG GTGTAGTTAT
2651  AAATTCTAAA TACTTTAATG TTTCAACAGG GTCAAGTTTA AGATTTAAAA
2701  CTTCAGGCTC AACAAAAACT GGCTTCTCAA TAGAGAAAGA TTTAACTTTA
2751  AATGCCACCG GAGGCAACAT AACACTTTTG CAAGTTGAAG GCACCGATGG
2801  AATGATTGGT AAAGGCATTG TAGCCAAAAA AAACATAACC TTTGAAGGAG
2851  GTAAGATGAG GTTTGGCTCC AGGAAAGCCG TAACAGAAAT CGAAGGCAAT
2901  GTTACTATCA ATAACAACGC TAACGTCACT CTTATCGGTT CGGATTTTGA
2951  CAACCATCAA AAACCTTTAA CTATTAAAAA AGATGTCATC ATTAATAGCG
3001  GCAACCTTAC CGCTGGAGGC AATATTGTCA ATATAGCCGG AAATCTTACC
3051  GTTGAAAGTA ACGCTAATTT CAAAGCTATC ACAAATTTCA CTTTTAATGT
3101  AGGCGGCTTG TTTGACAACA AAGGCAATTC AAATATTTCC ATTGCCAAAG
3151  GAGGGGCTCG CTTTAAAGAC ATTGATAATT CCAAGAATTT AAGCATCACC
3201  ACCAACTCCA GCTCCACTTA CCGCACTATT ATAAGCGGCA ATATAACCAA
3251  TAAAAACGGT GATTTAAATA TTACGAACGA AGGTAGTGAT ACTGAAATGC
```

FIG.6E.

3301	AAATTGGCGG	CGATGTCTCG	CAAAAGAAG	GTAATCTCAC	GATTTCTTCT
3351	GACAAAATCA	ATATTACCAA	ACAGATAACA	ATCAAGGCAG	GTGTTGATGG
3401	GGAGAATTCC	GATTCAGACG	CGACAAACAA	TGCCAATCTA	ACCATTAAAA
3451	CCAAAGAATT	GAAATTAACG	CAAGACCTAA	ATATTTCAGG	TTTCAATAAA
3501	GCAGAGATTA	CAGCTAAAGA	TGGTAGTGAT	TTAACTATTG	GTAACACCAA
3551	TAGTGCTGAT	GGTACTAATG	CCAAAAAAGT	AACCTTTAAC	CAGGTTAAAG
3601	ATTCAAAAAT	CTCTGCTGAC	GGTCACAAGG	TGACACTACA	CAGCAAAGTG
3651	GAAACATCCG	GTAGTAATAA	CAACACTGAA	GATAGCAGTG	ACAATAATGC
3701	CGGCTTAACT	ATCGATGCAA	AAAATGTAAC	AGTAAACAAC	AATATTACTT
3751	CTCACAAAGC	AGTGAGCATC	TCTGCGACAA	GTGGAGAAAT	TACCACTAAA
3801	ACAGGTACAA	CCATTAACGC	AACCACTGGT	AACGTGGAGA	TAACCGCTCA
3851	AACAGGTAGT	ATCCTAGGTG	GAATTGAGTC	CAGCTCTGGC	TCTGTAACAC
3901	TTACTGCAAC	CGAGGGCGCT	CTTGCTGTAA	GCAATATTTC	GGGCAACACC
3951	GTTACTGTTA	CTGCAAATAG	CGGTGCATTA	ACCACTTTGG	CAGGCTCTAC
4001	AATTAAAGGA	ACCGAGAGTG	TAACCACTTC	AAGTCAATCA	GGCGATATCG
4051	GCGGTACGAT	TTCTGGTGGC	ACAGTAGAGG	TTAAAGCAAC	CGAAAGTTTA

FIG.6F.

```
4101  ACCACTCAAT CCAATTCAAA AATTAAAGCA ACAACAGGCG AGGCTAACGT
4151  AACAAGTGCA ACAGGTACAA TTGGTGGTAC GATTTCCGGT AATACGGTAA
4201  ATGTTACGGC AAACGCTGGC GATTTAACAG TTGGGAATGG CGCAGAAATT
4251  AATGCGACAG AAGGAGCTGC AACCTTAACT ACATCATCGG GCAAATTAAC
4301  TACCGAAGCT AGTTCACACA TTACTTCAGC CAAGGGTCAG GTAAATCTTT
4351  CAGCTCAGGA TGGTAGCGTT GCAGGAAGTA TTAATGCCGC CAATGTGACA
4401  CTAAATACTA CAGGCACTTT AACTACCGTG AAGGGTTCAA ACATTAATGC
4451  AACCAGCGGT ACCTTGGTTA TTAACGCAAA AGACGCTGAG CTAAATGGCG
4501  CAGCATTGGG TAACCACACA GTGGTAAATG CAACCAACGC AAATGGCTCC
4551  GGCAGCGTAA TCGCGACAAC CTCAAGCAGA GTGAACATCA CTGGGGATTT
4601  AATCACAATA AATGGATTAA ATATCATTTC AAAAAACGGT ATAAACACCG
4651  TACTGTTAAA AGGCGTTAAA ATTGATGTGA AATACATTCA ACCGGGTATA
4701  GCAAGCGTAG ATGAAGTAAT TGAAGCGAAA CGCATCCTTG AGAAGGTAAA
4751  AGATTTATCT GATGAAGAAA GAGAAGCGTT AGCTAAACTT GGCGTAAGTG
4801  CTGTACGTTT TATTGAGCCA AATAATACAA TTACAGTCGA TACACAAAAT
4851  GAATTTGCAA CCAGACCATT AAGTCGAATA GTGATTTCTG AAGGCAGGGC
4901  GTGTTTCTCA AACAGTGATG GCGCGACGGT GTGCGTTAAT ATCGCTGATA
```

FIG.6G.

```
4951  ACGGGCGGTA GCGGTCAGTA ATTGACAAGG TAGATTTCAT CCTGCAATGA
5001  AGTCATTTTA TTTTCGTATT ATTTACTGTG TGGGTTAAAG TTCAGTACGG
5051  GCTTTACCCA TCTTGTAAAA AATTACGGAG AATACAATAA AGTATTTTTA
5101  ACAGGTTATT ATTATGAAAA ATATAAAAAG CAGATTAAAA CTCAGTGCAA
5151  TATCAGTATT GCTTGGCCTG GCTTCTTCAT CATTGTATGC AGAAGAAGCG
5201  TTTTTAGTAA AAGGCTTTCA GTTATCTGGT GCACTTGAAA CTTTAAGTGA
5251  AGACGCCCAA CTGTCTGTAG CAAAATCTTT ATCTAAATAC CAAGGCTCGC
5301  AAACTTTAAC AAACCTAAAA ACAGCACAGC TTGAATTACA GGCTGTGCTA
5351  GATAAGATTG AGCCAAATAA GTTTGATGTG ATATTGCCAC AACAAACCAT
5401  TACGGATGGC AATATTATGT TTGAGCTAGT CTCGAAATCA GCCGCAGAAA
5451  GCCAAGTTTT TTATAAGGCG AGCCAGGGTT ATAGTGAAGA AAATATCGCT
5501  CGTAGCCTGC CATCTTTGAA ACAAGGAAAA GTGTATGAAG ATGGTCGTCA
5551  GTGGTTCGAT TTGCGTGAAT TCAATATGGC AAAAGAAAAT CCACTTAAAG
5601  TCACTCGCGT GCATTACGAG TTAAACCCTA AAAACAAAAC CTCTGATTTG
5651  GTAGTTGCAG GTTTTTCGCC TTTTGGCAAA ACGCGTAGCT TTGTTTCCTA
5701  TGATAATTTC GGCGCAAGGG AGTTTAACTA TCAACGTGTA AGTCTAGGTT
```

FIG.6H.

```
5751  TTGTAAATGC CAATTTGACC GGACATGATG ATGTATTAAA TCTAAACGCA
5801  TTGACCAATG TAAAAGCACC ATCAAAATCT TATGCGGTAG GCATAGGATA
5851  TACTTATCCG TTTTATGATA AACACCAATC CTTAAGTCTT TATACCAGCA
5901  TGAGTTATGC TGATTCTAAT GATATCGACG GCTTACCAAG TGCGATTAAT
5951  CGTAAATTAT CAAAAGGTCA ATCTATCTCT GCGAATCTGA AATGGAGTTA
6001  TTATCTCCCG ACATTTAACC TTGGAATGGA AGACCAGTTT AAAATTAATT
6051  TAGGCTACAA CTACCGCCAT ATTAATCAAA CATCCGAGTT AAACACCCTG
6101  GGTGCAACGA AGAAAAAATT TGCAGTATCA GGCGTAAGTG CAGGCATTGA
6151  TGGACATATC CAATTTACCC CTAAAACAAT CTTTAATATT GATTTAACTC
6201  ATCATTATTA CGCGAGTAAA TTACCAGGCT CTTTTGGAAT GGAGCGCATT
6251  GGCGAAACAT TTAATCGCAG CTATCACATT AGCACAGCCA GTTTAGGGTT
6301  GAGTCAAGAG TTTGCTCAAG GTTGGCATTT TAGCAGTCAA TTATCGGGTC
6351  AGTTTACTCT ACAAGATATA AGTAGCATAG ATTTATTCTC TGTAACAGGT
6401  ACTTATGGCG TCAGAGGCTT TAAATACGGC GGTGCAAGTG GTGAGCGCGG
6451  TCTTGTATGG CGTAATGAAT TAAGTATGCC AAAATACACC CGCTTTCAAA
6501  TCAGCCCTTA TGCGTTTTAT GATGCAGGTC AGTTCCGTTA TAATAGCGAA
6551  AATGCTAAAA CTTACGGCGA AGATATGCAC ACGGTATCCT CTGCGGGTTT
```

FIG.6I.

```
6601  AGGCATTAAA ACCTCTCCTA CACAAAACTT AAGCTTAGAT GCTTTTGTTG
6651  CTCGTCGCTT TGCAAATGCC AATAGTGACA ATTTGAATGG CAACAAAAAA
6701  CGCACAAGCT CACCTACAAC CTTCTGGGGT AGATTAACAT TCAGTTTCTA
6751  ACCCTGAAAT TTAATCAACT GGTAAGCGTT CCGCCTACCA GTTTATAACT
6801  ATATGCTTTA CCCGCCAATT TACAGTCTAT ACGCAACCCT GTTTTCATCC
6851  TTATATATCA AACAAACTAA GCAAACCAAG CAAACCAAGC AAACCAAGCA
6901  AACCAAGCAA ACCAAGCAAA CCAAGCAAAC CAAGCAAACC AAGCAAACCA
6951  AGCAAACCAA GCAAACCAAG CAAACCAAGC AAACCAAGCA ATGCTAAAAA
7001  ACAATTTATA TGATAAACTA AAACATACTC CATACCATGG CAATACAAGG
7051  GATTTAATAA TATGACAAAA GAAAATTTAC AAAGTGTTCC ACAAAATACG
7101  ACCGCTTCAC TTGTAGAATC AAACAACGAC CAAACTTCCC TGCAAATACT
7151  TAAACAACCA CCCAAACCCA ACCTATTACG CCTGGAACAA CATGTCGCCA
7201  AAAAAGATTA TGAGCTTGCT TGCCGCGAAT TAATGGCGAT TTTGGAAAAA
7251  ATGGACGCTA ATTTTGGAGG CGTTCACGAT ATTGAATTTG ACGCACCTGC
7301  TCAGCTGGCA TATCTACCCG AAAAACTACT AATTCATTTT GCCACTCGTC
7351  TCGCTAATGC AATTACAACA CTCTTTTCCG ACCCCGAATT GGCAATTTCC
```

FIG.6J.

```
7401  GAAGAAGGGG CATTAAAGAT GATTAGCCTG CAACGCTGGT TGACGCTGAT
7451  TTTTGCCTCT TCCCCCTACG TTAACGCAGA CCATATTCTC AATAAATATA
7501  ATATCAACCC AGATTCCGAA GGTGGCTTTC ATTTAGCAAC AGACAACTCT
7551  TCTATTGCTA AATTCTGTAT TTTTTACTTA CCCGAATCCA ATGTCAATAT
7601  GAGTTTAGAT GCGTTATGGG CAGGGAATCA ACAACTTTGT GCTTCATTGT
7651  GTTTTGCGTT GCAGTCTTCA CGTTTTATTG GTACTGCATC TGCGTTTCAT
7701  AAAAGAGCGG TGGTTTTACA GTGGTTTCCT AAAAAACTCG CCGAAATTGC
7751  TAATTTAGAT GAATTGCCTG CAAATATCCT TCATGATGTA TATATGCACT
7801  GCAGTTATGA TTTAGCAAAA AACAAGCACG ATGTTAAGCG TCCATTAAAC
7851  GAACTTGTCC GCAAGCATAT CCTCACGCAA GGATGGCAAG ACCGCTACCT
7901  TTACACCTTA GGTAAAAAGG ACGGCAAACC TGTGATGATG GTACTGCTTG
7951  AACATTTTAA TTCGGGACAT TCGATTTATC GCACGCATTC AACTTCAATG
8001  ATTGCTGCTC GAGAAAAATT CTATTTAGTC GGCTTAGGCC ATGAGGGCGT
8051  TGATAACATA GGTCGAGAAG TGTTTGACGA GTTCTTTGAA ATCAGTAGCA
8101  ATAATATAAT GGAGAGACTG TTTTTTATCC GTAAACAGTG CGAAACTTTC
8151  CAACCCGCAG TGTTCTATAT GCCAAGCATT GGCATGGATA TTACCACGAT
```

FIG.6K.

```
8201  TTTTGTGAGC AACACTCGGC TTGCCCCTAT TCAAGCTGTA GCCTTGGGTC
8251  ATCCTGCCAC TACGCATTCT GAATTTATTG ATTATGTCAT CGTAGAAGAT
8301  GATTATGTGG GCAGTGAAGA TTGTTTAGC  GAAACCCTTT TACGCTTACC
8351  CAAAGATGCC CTACCTTATG TACCATCTGC ACTCGCCCCA CAAAAAGTGG
8401  ATTATGTACT CAGGGAAAAC CCTGAAGTAG TCAATATCGG TATTGCCGCT
8451  ACCACAATGA AATTAAACCC TGAATTTTTG CTAACATTGC AAGAAATCAG
8501  AGATAAAGCT AAAGTCAAAA TACATTTTCA TTTCGCACTT GGACAATCAA
8551  CAGGCTTGAC ACACCCTTAT GTCAAATGGT TTATCGAAAG CTATTTAGGT
8601  GACGATGCCA CTGCACATCC CCACGCACCT TATCACGATT ATCTGGCAAT
8651  ATTGCGTGAT TGCGATATGC TACTAAATCC GTTTCCTTTC GGTAATACTA
8701  ACGGCATAAT TGATATGGTT ACATTAGGTT TAGTTGGTGT ATGCAAAACG
8751  GGGGATGAAG TACATGAACA TATTGATGAA GGTCTGTTTA AACGCTTAGG
8801  ACTACCAGAA TGGCTGATAG CCGACACACG AGAAACATAT ATTGAATGTG
8851  CTTTGCGTCT AGCAGAAAAC CATCAAGAAC GCCTTGAACT CCGTCGTTAC
8901  ATCATAGAAA ACAACGGCTT ACAAAAGCTT TTTACAGGCG ACCCTCGTCC
8951  ATTGGGCAAA ATACTGCTTA AGAAAACAAA TGAATGGAAG CGGAAGCACT
9001  TGAGTAAAAA ATAACGGTTT TTTAAAGTAA AAGTGCGGTT AATTTTCAAA
```

FIG.6L.

```
9051  GCGTTTTAAA AACCTCTCAA AAATCAACCG CACTTTTATC TTTATAACGC
9101  TCCCCGCGCG CTGACAGTTTA TCTCTTTCTT AAAATACCCA TAAAATTGTG
9151  GCAATAGTTG GGTAATCAAA TTCAATTGTT GATACGGCAA ACTAAAGACG
9201  GCGCGTTCTT CGGCAGTCAT C
```

FIG.7A.

```
  1  CGCCACTTCA ATTTTGGATT GTTGAAATTC AACTAACCAA AAAGTGCGGT
 51  TAAAATCTGT GGAGAAAATA GGTTGTAGTG AAGAACGAGG TAATTGTTCA
101  AAAGGATAAA GCTCTCTTAA TTGGGCATTG GTTGGCGTTT CTTTTTCGGT
151  TAATAGTAAA TTATATTCTG GACGACTATG CAATCCACCA ACAACTTTAC
201  CGTTGGTTTT AAGCGTTAAT GTAAGTTCTT GCTCTTCTTG GCGAATACGT
251  AATCCCATTT TTTGTTTAGC AAGAAAATGA TCGGGATAAT CATAATAGGT
301  GTTGCCCAAA AATAAATTTT GATGTTCTAA AATCATAAAT TTTGCAAGAT
351  ATTGTGGCAA TTCAATACCT ATTTGTGGCG AAATCGCCAA TTTTAATTCA
401  ATTTCTTGTA GCATAATATT TCCCACTCAA ATCAACTGGT TAAATATACA
451  AGATAATAAA AATAAATCAA GATTTTTGTG ATGACAAACA ACAATTACAA
501  CACCTTTTTT GCAGTCTATA TGCAAATATT TTAAAAAAAT AGTATAAATC
551  CGCCATATAA AATGGTATAA TCTTTCATCT TTCATCTTTC ATCTTTCATC
601  TTTCATCTTT CATCTTTCAT CTTTCATCTT TCATCTTTCA TCTTTCATCT
651  TTCATCTTTC ATCTTTCATC TTTCATCTTT CACATGAAAT GATGAACCGA
701  GGGAAGGGAG GGAGGGGCAA GAATGAAGAG GGAGCTGAAC GAACGCAAAT
751  GATAAAGTAA TTTAATTGTT CAACTAACCT TAGGAGAAAA TATGAACAAG
```

FIG.7B.

```
 801  ATATATCGTC TCAAATTCAG CAAACGCCTG AATGCTTTGG TTGCTGTGTC
 851  TGAATTGGCA CGGGGTTGTG ACCATTCCAC AGAAAAAGGC AGCGAAAAAC
 901  CTGCTCGCAT GAAAGTGCGT CACTTAGCGT TAAAGCCACT TTCCGCTATG
 951  TTACTATCTT TAGGTGTAAC ATCTATTCCA CAATCTGTTT TAGCAAGCGG
1001  CAATTTAACA TCGACCAAAA TGAAATGGTG CAGTTTTTAC AAGAAAACAA
1051  GTAATAAAAC CATTATCCGC AACAGTGTTG ACGCTATCAT TAATTGGAAA
1101  CAATTTAACA TCGACCAAAA TGAAATGGTG CAGTTTTTAC AAGAAAACAA
1151  CAACTCCGCC GTATTCAACC GTGTTACATC TAACCAAATC TCCCAATTAA
1201  AAGGGATTTT AGATTCTAAC GGACAAGTCT TTTTAATCAA CCCAAATGGT
1251  ATCACAATAG GTAAAGACGC AATTATTAAC ACTAATGGCT TTACGGCTTC
1301  TACGCTAGAC ATTTCTAACG AAAACATCAA GGCGCGTAAT TTCACCTTCG
1351  AGCAAACCAA AGATAAAGCG CTCGCTGAAA TTGTGAATCA CGGTTTAATT
1401  ACTGTCGGTA AAGACGGCAG TGTAAATCTT ATTGGTGGCA AAGTGAAAAA
1451  CGAGGGTGTG ATTAGCGTAA ATGGTGGCAG CATTTCTTTA CTCGCAGGGC
1501  AAAAAATCAC CATCAGCGAT ATAATAAACC CAACCATTAC TTACAGCATT
1551  GCCGCGCCTG AAAATGAAGC GGTCAATCTG GGCGATATTT TTGCCAAAGG
```

FIG.7C.

```
1601  CGGTAACATT AATGTCCGTG CTGCCACTAT TCGAAACCAA GGTAAACTTT
1651  CTGCTGATTC TGTAAGCAAA GATAAAAGCG GCAATATTGT TCTTTCCGCC
1701  AAAGAGGGTG AAGCGGAAAT TGGCGGTGTA ATTTCCGCTC AAAATCAGCA
1751  AGCTAAAGGC GGCAAGCTGA TGATTACAGG CGATAAAGTC ACATTAAAAA
1801  CAGGTGCAGT TATCGACCTT TCAGGTAAAG AAGGGGGAGA AACTTACCTT
1851  GGCGGTGACG AGCGCGGCGA AGGTAAAAAC GGCATTCAAT TAGCAAAGAA
1901  AACCTCTTTA GAAAAAGGCT CAACCATCAA TGTATCAGGC AAAGAAAAAG
1951  GCGGACGCGC TATTGTGTGG GGCGATATTG CGTTAATTGA CGGCAATATT
2001  AACGCTCAAG GTAGTGGTGA TATCGCTAAA ACCGGTGGTT TTGTGGAGAC
2051  ATCGGGGCAT TATTTATCCA TTGACAGCAA TGCAATTGTT AAAACAAAAG
2101  AGTGGTTGCT AGACCCTGAT GATGTAACAA TTGAAGCCGA AGACCCCCTT
2151  CGCAATAATA CCGGTATAAA TGATGAATTC CCAACAGGCA CCGGTGAAGC
2201  AAGCGACCCT AAAAAAAATA GCGAACTCAA AACAACGCTA ACCAATACAA
2251  CTATTTCAAA TTATCTGAAA AACGCCTGGA CAATGAATAT AACGGCATCA
2301  AGAAAACTTA CCGTTAATAG CTCAATCAAC ATCGGAAGCA ACTCCCACTT
2351  AATTCTCCAT AGTAAAGGTC AGCGTGGCGG AGGCGTTCAG ATTGATGGAG
2401  ATATTACTTC TAAAGGCGGA AATTTAACCA TTTATTCTGG CGGATGGGTT
```

FIG.7D.

```
2451  GATGTTCATA AAAATATTAC GCTTGATCAG GGTTTTTTAA ATATTACCGC
2501  CGCTTCCGTA GCTTTTGAAG GTGGAAATAA CAAAGCACGC GACGCGGCAA
2551  ATGCTAAAAT TGTCGCCCAG GGCACTGTAA CCATTACAGG AGAGGGAAAA
2601  GATTTCAGGG CTAACAACGT ATCTTTAAAC GGAACGGGTA AAGGTCTGAA
2651  TATCATTTCA TCAGTGAATA ATTTAACCCA CAATCTTAGT GGCACAATTA
2701  ACATATCTGG GAATATAACA ATTAACCAAA CTACGAGAAA GAACACCTCG
2751  TATTGGCAAA CCAGCCATGA TTCGCACTGG AACGTCAGTG CTCTTAATCT
2801  AGAGACAGGC GCAAATTTTA CCTTTATTAA ATACATTTCA AGCAATAGCA
2851  AAGGCTTAAC AACACAGTAT AGAAGCTCTG CAGGGGTGAA TTTTAACGGC
2901  GTAAATGGCA ACATGTCATT CAATCTCAAA GAAGGAGCGA AAGTTAATTT
2951  CAAATTAAAA CCAAACGAGA ACATGAACAC AAGCAAACCT TTACCAATTC
3001  GGTTTTTAGC CAATATCACA GCCACTGGTG GGGGCTCTGT TTTTTTTGAT
3051  ATATATGCCA ACCATTCTGG CAGAGGGGCT GAGTTAAAAA TGAGTGAAAT
3101  TAATATCTCT AACGGCGCTA ATTTTACCTT AAATTCCCAT GTTCGCGGCG
3151  ATGACGCTTT TAAAATCAAC AAAGACTTAA CCATAAATGC AACCAATTCA
3201  AATTTCAGCC TCAGACAGAC GAAAGATGAT TTTTATGACG GGTACGCACG
```

FIG.7E.

```
3251  CAATGCCATC AATTCAACCT ACAACATATC CATTCTGGGC GGTAATGTCA
3301  CCCTTGGTGG ACAAAACTCA AGCAGCAGCA TTACGGGGAA TATTACTATC
3351  GAGAAAGCAG CAAATGTTAC GCTAGAAGCC AATAACGCCC CTAATCAGCA
3401  AAACATAAGG GATAGAGTTA TAAAACTTGG CAGCTTGCTC GTTAATGGGA
3451  GTTTAAGTTT AACTGGCGAA AATGCAGATA TTAAAGGCAA TCTCACTATT
3501  TCAGAAAGCG CCACTTTTAA AGGAAAGACT AGAGATACCC TAAATATCAC
3551  CGGCAATTTT ACCAATAATG GCACTGCCGA AATTAATATA ACACAAGGAG
3601  TGGTAAAACT TGGCAATGTT ACCAATGATG GTGATTTAAA CATTACCACT
3651  CACGCTAAAC GCAACCAAAG AAGCATCATC GGCGGAGATA TAATCAACAA
3701  AAAAGGAAGC TTAAATATTA CAGACAGTAA TAATGATGCT GAAATCCAAA
3751  TTGGCGGCAA TATCTCGCAA AAAGAAGGCA ACCTCACGAT TTCTTCCGAT
3801  AAAATTAATA TCACCAAACA GATAACAATC AAAAAGGGTA TTGATGGAGA
3851  GGACTCTAGT TCAGATGCGA CAAGTAATGC CAACCTAACT ATTAAAACCA
3901  AAGAATTGAA ATTGACAGAA GACCTAAGTA TTTCAGGTTT CAATAAAGCA
3951  GAGATTACAG CCAAAGATGG TAGAGATTTA ACTATTGGCA ACAGTAATGA
4001  CGGTAACAGC GGTGCCGAAG CCAAAACAGT AACTTTTAAC AATGTTAAAG
```

FIG.7F.

```
4051  ATTCAAAAAT CTCTGCTGAC GGTCACAATG TGACACTAAA TAGCAAAGTG
4101  AAAACATCTA GCAGCAATGG CGGACGTGAA AGCAATAGCG ACAACGATAC
4151  CGGCTTAACT ATTACTGCAA AAAATGTAGA AGTAAACAAA GATATTACTT
4201  CTCTCAAAAC AGTAAATATC ACCGCGTCGG AAAAGGTTAC CACCACAGCA
4251  GGCTCGACCA TTAACGCAAC AAATGGCAAA GCAAGTATTA CAACCAAAAC
4301  AGGTGATATC AGCGGTACGA TTTCCGGTAA CACGGTAAGT GTTAGCGCGA
4351  CTGGTGATTT AACCACTAAA TCCGGCTCAA AAATTGAAGC GAAATCGGGT
4401  GAGGCTAATG TAACAAGTGC AACAGGTACA ATTGGCGGTA CAATTTCCGG
4451  TAATACGGTA AATGTTACGG CAAACGCTGG CGATTTAACA GTTGGGAATG
4501  GCGCAGAAAT TAATGCGACA GAAGGAGCTG CAACCTTAAC CGCAACAGGG
4551  AATACCTTGA CTACTGAAGC CGGTTCTAGC ATCACTTCAA CTAAGGGTCA
4601  GGTAGACCTC TTGGCTCAGA ATGGTAGCAT CGCAGGAAGC ATTAATGCTG
4651  CTAATGTGAC ATTAAATACT ACAGGCACCT TAACCACCGT GGCAGGCTCG
4701  GATATTAAAG CAACCAGCGG CACCTTGGTT ATTAACGCAA AAGATGCTAA
4751  GCTAAATGGT GATGCATCAG GTGATAGTAC AGAAGTGAAT GCAGTCAACG
4801  ACTGGGGATT TGGTAGTGTG ACTGCGGCAA CCTCAAGCAG TGTGAATATC
4851  ACTGGGGATT TAAACACAGT AAATGGGTTA AATATCATTT CGAAAGATGG
```

FIG.7G.

```
4901  TAGAAACACT GTGCGCTTAA GAGGCAAGGA AATTGAGGTG AAATATATCC
4951  AGCCAGGTGT AGCAAGTGTA GAAGAAGTAA TTGAAGCGAA ACGCGTCCTT
5001  GAAAAAGTAA AAGATTTATC TGATGAAGAA AGAGAAACAT TAGCTAAACT
5051  TGGTGTAAGT GCTGTACGTT TTGTTGAGCC AAATAATACA ATTACAGTCA
5101  ATACACAAAA TGAATTTACA ACCAGACCGT CAAGTCAAGT GATAATTTCT
5151  GAAGGTAAGG CGTGTTTCTC AAGTGGTAAT GGCGCACGAG TATGTACCAA
5201  TGTTGCTGAC GATGGACAGC CGTAGTCAGT AATTGACAAG GTAGATTTCA
5251  TCCTGCAATG AAGTCATTTT ATTTTCGTAT TATTTACTGT GTGGGTTAAA
5301  GTTCAGTACG GGCTTTACCC ATCTTGTAAA AAATTACGGA GAATACAATA
5351  AAGTATTTTT AACAGGTTAT TATTATGAAA AATATAAAAA GCAGATTAAA
5401  ACTCAGTGCA ATATCAGTAT TGCTTGGCCT GGCTTCTTCA TCATTGTATG
5451  CAGAAGAAGC GTTTTTAGTA AAAGGCTTTC AGTTATCTGG TGCACTTGAA
5501  ACTTTAAGTG AAGACGCCCA ACTGTCTGTA GCAAAATCTT TATCTAAATA
5551  CCAAGGCTCG CAAACTTTAA CAAACCTAAA AACAGCACAG CTTGAATTAC
5601  AGGCTGTGCT AGATAAGATT GAGCCAAATA AATTTGATGT GATATTGCCG
5651  CAACAAACCA TTACGGATGG CAATATCATG TTTGAGCTAG TCTCGAAATC
```

FIG.7H.

```
5701  AGCCGCAGAA AGCCAAGTTT TTTATAAGGC GAGCCAGGGT TATAGTGAAG
5751  AAAATATCGC TCGTAGCCTG CCATCTTTGA AACAAGGAAA AGTGTATGAA
5801  GATGGTCGTC AGTGGTTCGA TTTGCGTGAA TTTAATATGG CAAAAGAAAA
5851  CCCGCTTAAG GTTACCCGTG TACATTACGA ACTAAACCCT AAAAACAAAA
5901  CCTCTAATTT GATAATTGCG GGCTTCTCGC CTTTTGGTAA AACGCGTAGC
5951  TTTATTTCTT ATGATAATTT CGGCGCGAGA GAGTTTAACT ACCAACGTGT
6001  AAGCTTGGGT TTTGTTAATG CCAATTTAAC TGGTCATGAT GATGTGTTAA
6151  TTATACCAGT ATGAGTTATG CTGATTCTAA TGATATCGAC GGCTTACCAA
6201  GTGCGATTAA TCGTAAATTA TCAAAAGGTC AATCTATCTC TGCGAATCTG
6251  AAATGGAGTT ATTATCTCCC AACATTTAAC CTTGGCATGG AAGACCAATT
6301  TAAAATTAAT TTAGGCTACA ACTACCGCCA TATTAATCAA ACCTCCGCGT
6351  TAAATCGCTT GGGTGAAACG AAGAAAAAAT TTGCAGTATC AGGCGTAAGT
6401  GCAGGCATTG ATGGACATAT CCAATTTACC CCTAAAACAA TCTTTAATAT
6451  TGATTTAACT CATCATTATT ACGCGAGTAA ATTACCAGGC TCTTTTGGAA
6501  TGGAGCGCAT TGGCGAAACA TTTAATCGCA GCTATCACAT TAGCACAGCC
6551  AGTTTAGGGT TGAGTCAAGA GTTTGCTCAA GGTTGGCATT TTAGCAGTCA
6601  ATTATCAGGT CAATTTACTC TACAAGATAT TAGCAGTATA GATTTATTCT
```

FIG.7I.

```
6651  CTGTAACAGG TACTTATGGC GTCAGAGGCT TTAAATACGG CGGTGCAAGT
6701  GGTGAGCGCG GTCTTGTATG GCGTAATGAA TTAAGTATGC CAAAATACAC
6751  CCGCTTCCAA ATCAGCCCTT ATGCGTTTTA TGATGCAGGT CAGTTCCGTT
6801  ATAATAGCGA AAATGCTAAA ACTTACGGCG AAGATATGCA CACGGTATCC
6851  TCTGCGGGTT TAGGCATTAA AACCTCTCCT ACACAAAACT TAAGCCTAGA
6901  TGCTTTTGTT GCTCGTCGCT TTGCAAATGC CAATAGTGAC AATTTGAATG
6951  GCAACAAAAA ACGCACAAGC TCACCTACAA CCTTCTGGGG GAGATTAACA
7001  TTCAGTTTCT AACCCTGAAA TTTAATCAAC TGGTAAGCGT TCCGCCTACC
7051  AGTTTATAAC TATATGCTTT ACCCGCCAAT TTACAGTCTA TAGGCAACCC
7101  TGTTTTTACC CTTATATATC AAATAAACAA GCTAAGCTGA GCTAAGCAAA
7151  CCAAGCAAAC TCAAGCAAGC CAAGTAATAC TAAAAAAACA ATTTATATGA
7201  TAAACTAAAG TATACTCCAT GCCATGGCGA TACAAGGGAT TTAATAATAT
7251  GACAAAAGAA AATTTGCAAA ACGCTCCTCA AGATGCGACC GCTTTACTTG
7301  CGGAATTAAG CAACAATCAA ACTCCCCTGC GAATATTTAA ACAACCACGC
7351  AAGCCCAGCC TATTACGCTT GGAACAACAT ATCGCAAAAA AAGATTATGA
7401  GTTTGCTTGT CGTGAATTAA TGGTGATTCT GGAAAAAATG GACGCTAATT
```

FIG.7J.

```
7451  TTGGAGGCGT TCACGATATT GAATTTGACG CACCCGCTCA GCTGGCATAT
7501  CTACCCGAAA AATTACTAAT TTATTTTGCC ACTCGTCTCG CTAATGCAAT
7551  TACAACACTC TTTTCCGACC CCGAATTGGC AATTTCTGAA GAAGGGGCGT
7601  TAAAGATGAT TAGCCTGCAA CGCTGGTTGA CGCTGATTTT TGCCTCTTCC
7651  CCCTACGTTA ACGCAGACCA TATTCTCAAT AAATATAATA TCAACCCAGA
7701  TTCCGAAGGT GGCTTTCATT TAGCAACAGA CAACTCTTCT ATTGCTAAAT
7751  TCTGTATTTT TTACTTACCC GAATCCAATG TCAATATGAG TTTAGATGCG
7801  TTATGGGCAG GGAATCAACA ACTTTGTGCT TCATTGTGTT TTGCGTTGCA
7851  GTCTTCACGT TTTATTGGTA CCGCATCTGC GTTTCATAAA AGAGCGGTGG
7901  TTTTACAGTG GTTTCCTAAA AAACTCGCCG AAATTGCTAA TTTAGATGAA
7951  TTGCCTGCAA ATATCCTTCA TGATGTATAT ATGCACTGCA GTTATGATTT
8001  AGCAAAAAAC AAGCACGATG TTAAGCGTCC ATTAAACGAA CTTGTCCGCA
8051  AGCATATCCT CACGCAAGGA TGGCAAGACC GCTACCTTTA CACCTTAGGT
8101  AAAAAGGACG GCAAACCTGT GATGATGGTA CTGCTTGAAC ATTTTAATTC
8151  GGGACATTCG ATTTATCGTA CACATTCAAC TTCAATGATT GCTGCTCGAG
8201  AAAAATTCTA TTTAGTCGGC TTAGGCCATG AGGGCGTTGA TAAAATAGGT
```

FIG.7K.

```
8251  CGAGAAGTGT TTGACGAGTT CTTTGAAATC AGTAGCAATA ATATAATGGA
8301  GAGACTGTTT TTTATCCGTA AACAGTGCGA AACTTTCCAA CCCGCAGTGT
8351  TCTATATGCC AAGCATTGGC ATGGATATTA CCACGATTTT TGTGAGCAAC
8401  ACTCGGCTTG CCCCTATTCA AGCTGTAGCC CTGGGTCATC CTGCCACTAC
8451  GCATTCTGAA TTTATTGATT ATGTCATCGT AGAAGATGAT TATGTGGGCA
8501  GTGAAGATTG TTTCAGCGAA ACCCTTTTAC GCTTACCCAA AGATGCCCTA
8551  CCTTATGTAC CTTCTGCACT CGCCCCACAA AAAGTGGATT ATGTACTCAG
8601  GGAAAACCCT GAAGTAGTCA ATATCGGTAT TGCCGCTACC ACAATGAAAT
8651  TAAACCCTGA ATTTTTGCTA ACATTGCAAG AAATCAGAGA TAAAGCTAAA
8701  GTCAAAATAC ATTTTCATTT CGCACTTGGA CAATCAACAG GCTTGACACA
8751  CCCTTATGTC AAATGGTTTA TCGAAAGCTA TTTAGGTGAC GATGCCACTG
8801  CACATCCCCA CGCACCTTAT CACGATTATC TGGCAATATT GCGTGATTGC
8851  GATATGCTAC TAAATCCGTT TCCTTTCGGT AATACTAACG GCATAATTGA
8901  TATGGTTACA TTAGGTTTAG TTGGTGTATG CAAAACGGGG GATGAAGTAC
8951  ATGAACATAT TGATGAAGGT CTGTTTAAAC GCTTAGGACT ACCAGAATGG
9001  CTGATAGCCG ACACACGAGA AACATATATT GAATGTGCTT TGCGTCTAGC
9051  AGAAAACCAT CAAGAACGCC TTGAACTCCG TCGTTACATC ATAGAAAACA
```

FIG. 7L.

```
9101  ACGGCTTACA AAAGCTTTTT ACAGGCGACC CTCGTCCATT GGGCAAAATA
9151  CTGCTTAAGA AAACAAATGA ATGGAAGCGG AAGCACTTGA GTAAAAAATA
9201  ACGGTTTTTT AAAGTAAAAG TGCGGTTAAT TTTCAAAGCG TTTTAAAAAC
9251  CTCTCAAAAA TCAACCGCAC TTTTATCTTT ATAACGATCC CGCACGCTGA
9301  CAGTTTATCA GCCTCCCCGCC ATAAAACTCC GCCTTTCATG GCGGAGATTT
9351  TAGCCAAAAC TGGCAGAAAT TAAAGGCTAA AATCACCAAA TTGCACCACA
9401  AAATCACCAA TACCCACAAA AAA
```

FIG.8A.

```
  1  GATCAATCTG GGCGATATTT TTGCCAAAGG TGGTAACATT AATGTCCGCG
 51  CTGCCACTAT TCGCAATAAA GGTAAACTTT CTGCCGACTC TGTAAGCAAA
101  GATAAAAGTG GTAACATTGT TCTCTCTGCC AAAGAAGGTG AAGCGGAAAT
151  TGGCGGTGTA ATTTCCGCTC AAAATCAGCA AGCCAAAGGT GGTAAGTTGA
201  TGATTACAGG CGATAAAGTT ACATTGAAAA CGGGTGCAGT TATCGACCTT
251  TCGGGTAAAG AAGGGGGAGA AACTTATCTT GGCGGTGACG AGCGTGGCGA
301  AGGTAAAAAC GGCATTCAAT TAGCAAAGAA AACCACTTTA GAAAAAGGCT
351  CAACAATTAA TGTGTCAGGT AAAGAAAAAG GTGGGCGCGC TATTGTATGG
401  GGCGATATTG CGTTAATTGA CGGCAATATT AATGCCCAAG GTAAAGATAT
451  CGCTAAAACT GGTGGTTTTG TGGAGACGTC GGGGCATTAC TTATCCATTG
501  ATGATAACGC AATTGTTAAA ACAAAAGAAT GGCTACTAGA CCCAGAGAAT
551  GTGACTATTG AAGCTCCTTC CGCTTCTCGC GTCGAGCTGG GTGCCGATAG
601  GAATTCCCAC TCGGCAGAGG TGATAAAAGT GACCCTAAAA AAAAATAACA
651  CCTCCTTGAC AACACTAACC AATACAACCA TTTCAAATCT TCTGAAAAGT
701  GCCCACGTGG TGAACATAAC GGCAAGGAGA AAACTTACCG TTAATAGCTC
751  TATCAGTATA GAAAGAGGCT CCCACTTAAT TCTCCACAGT GAAGGTCAGG
```

FIG.8B.

```
 801  GCGGTCAAGG TGTTCAGATT GATAAAGATA TTACTTCTGA AGGCGGAAAT
 851  TTAACCATTT ATTCTGGCGG ATGGGTTGAT GTTCATAAAA ATATTACGCT
 901  TGGTAGCGGC TTTTTAAACA TCACAACTAA AGAAGGAGAT ATCGCCTTCG
 951  AAGACAAGTC TGGACGGAAC AACCTAACCA TTACAGCCCA AGGGACCATC
1001  ACCTCAGGTA ATAGTAACGG CTTTAGATTT AACAACGTCT CTCTAAACAG
1051  CCTTGGCGGA AAGCTGAGCT TTACTGACAG CAGAGAGGAC AGAGGTAGAA
1101  GAACTAAGGG TAATATCTCA AACAAATTTG ACGGAACGTT AAACATTTCC
1151  GGAACTGTAG ATATCTCAAT GAAAGCACCC AAAGTCAGCT GGTTTTACAG
1201  AGACAAAGGA CGCACCTACT GGAACGTAAC CACTTTAAAT GTTACCTCGG
1251  GTAGTAAATT TAACCTCTCC ATTGACAGCA CAGGAAGTGG CTCAACAGGT
1301  CCAAGCATAC GCAATGCAGA ATTAAATGGC ATAACATTTA ATAAAGCCAC
1351  TTTTAATATC GCACAAGGCT CAACAGCTAA CTTTAGCATC AAGGCATCAA
1401  TAATGCCCTT TAAGAGTAAC GCTAACTACG CATTATTTAA TGAAGATATT
1451  TCAGTCTCAG GGGGGGGTAG CGTTAATTTC AAACTTAACG CCTCATCTAG
1501  CAACATACAA ACCCCTGGCG TAATTATAAA ATCTCAAAAC TTTAATGTCT
1551  CAGGAGGGTC AACTTTAAAT CTCAAGGCTG AAGGTTCAAC AGAAACCGCT
1601  TTTTCAATAG AAAATGATTT AAACTTAAAC GCCACCGGTG GCAATATAAC
```

FIG.8C.

```
1651  AATCAGACAA GTCGAGGGTA CCGATTCACG CGTCAACAAA GGTGTCGCAG
1701  CCAAAAAAAA CATAACTTTT AAAGGGGGTA ATATCACCTT CGGCTCTCAA
1751  AAAGCCACAA CAGAAATCAA AGGCAATGTT ACCATCAATA AAAACACTAA
1801  CGCTACTCTT CGTGGTGCGA ATTTTGCCGA AAACAAATCG CCTTTAAATA
1851  TAGCAGGAAA TGTTATTAAT AATGGCAACC TTACCACTGC CGGCTCCATT
1901  ATCAATATAG CCGGAAATCT TACTGTTTCA AAAGGCGCTA ACCTTCAAGC
1951  TATAACAAAT TACACTTTTA ATGTAGCCGG CTCATTTGAC AACAATGGCG
2001  CTTCAAACAT TTCCATTGCC AGAGGAGGGG CTAAATTTAA AGATATCAAT
2051  AACACCAGTA GCTTAAATAT TACCACCAAC TCTGATACCA CTTACCGCAC
2101  CATTATAAAA GGCAATATAT CCAACAAATC AGGTGATTTG AATATTATTG
2151  ATAAAAAAAG CGACGCTGAA ATCCAAATTG GCGGCAATAT CTCACAAAAA
2201  GAAGGCAATC TCACAATTTC TTCTGATAAA GTAAATATTA CCAATCAGAT
2251  AACAATCAAA GCAGGCGTTG AAGGGGGGCG TTCTGATTCA AGTGAGGCAG
2301  AAAATGCTAA CCTAACTATT CAAACCAAAG AGTTAAAATT GGCAGGAGAC
2351  CTAAATATTT CAGGCTTTAA TAAAGCAGAA ATTACAGCTA AAATGGCAG
2401  TGATTTAACT ATTGGCAATG CTAGCGGTGG TAATGCTGAT GCTAAAAAAG
```

FIG.8D.

```
2451  TGACTTTTGA CAAGGTTAAA GATTCAAAAA TCTCGACTGA CGGTCACAAT
2501  GTAACACTAA ATAGCGAAGT GAAAACGTCT AATGGTAGTA GCAATGCTGG
2551  TAATGATAAC AGCACCGGTT TAACCATTTC CGCAAAAGAT GTAACGGTAA
2601  ACAATAACGT TACCTCCCAC AAGACAATAA ATATCTCTGC CGCAGCAGGA
2651  AATGTAACAA CCAAAGAAGG CACAACTATC AATGCAACCA CAGGCAGCGT
2701  GGAAGTAACT GCTCAAAATG GTACAATTAA AGGCAACATT ACCTCGCAAA
2751  ATGTAACAGT GACAGCAACA GAAAATCTTG TTACCACAGA GAATGCTGTC
2801  ATTAATGCAA CCAGCGGCAC AGTAAACATT AGTACAAAAA CAGGGGATAT
2851  TAAAGGTGGA ATTGAATCAA CTTCCGGTAA TGTAAATATT ACAGCGAGCG
2901  GCAATACACT TAAGGTAAGT AATATCACTG GTCAAGATGT AACAGTAACA
2951  GCGGATGCAG GAGCCTTGAC AACTACAGCA GGCTCAACCA TTAGTGCGAC
3001  AACAGGCAAT GCAAATATTA CAACCAAAAC AGGTGATATC AACGGTAAAG
3051  TTGAATCCAG CTCCGGCTCT GTAACACTTG TTGCAACTGG AGCAACTCTT
3101  GCTGTAGGTA ATATTTCAGG TAACACTGTT ACTATTACTG CGGATAGCGG
3151  TAAATTAACC TCCACAGTAG GTTCTACAAT TAATGGGACT AATAGTGTAA
3201  CCACCTCAAG CCAATCAGGC GATATTGAAG GTACAATTTC TGGTAATACA
3251  GTAAATGTTA CAGCAAGCAC TGGTGATTTA ACTATTGGAA ATAGTGCAAA
```

FIG.8E.

```
3301  AGTTGAAGCG AAAAATGGAG CTGCAACCTT AACTGCTGAA TCAGGCAAAT
3351  TAACCACCCA AACAGGCTCT AGCATTACCT CAAGCAATGG TCAGACAACT
3401  CTTACAGCCA AGGATAGCAG TATCGCAGGA AACATTAATG CTGCTAATGT
3451  GACGTTAAAT ACCACAGGCA CTTTAACTAC TACAGGGGAT TCAAAGATTA
3501  ACGCAACCAG TGGTACCTTA ACAATCAATG CAAAAGATGC CAAATTAGAT
3551  GGTGCTGCAT CAGGTGACCG CACAGTAGTA AATGCAACTA ACGCAAGTGG
3601  CTCTGGTAAC GTGACTGCGA AAACCTCAAG CAGCGTGAAT ATCACCGGGG
3651  ATTTAAACAC AATAAATGGG TTAAATATCA TTTCGGAAAA TGGTAGAAAC
3701  ACTGTGCGCT TAAGAGGCAA GGAAATTGAT GTGAAATATA TCCAACCAGG
3751  TGTAGCAAGC GTAGAAGAGG TAATTGAAGC GAAACGCGTC CTTGAGAAGG
3801  TAAAAGATTT ATCTGATGAA GAAAGAGAAA CACTAGCCAA ACTTGGTGTA
3851  AGTGCTGTAC GTTTCGTTGA GCCAAATAAT GCCATTACGG TTAATACACA
3901  AAACGAGTTT ACAACCAAAC CATCAAGTCA AGTGACAATT TCTGAAGGTA
3951  AGGCGTGTTT CTCAAGTGGT AATGGCGCAC GAGTATGTAC CAATGTTGCT
4001  GACGATGGAC AGCAGTAGTC AGTAATTGAC AAGGTAGATT TCATCCTGCA
4051  ATGAAGTCAT TTTATTTTCG TATTATTTAC TGTGTGGGTT AAAGTTCAGT
```

FIG.8F.

```
4101  ACGGGCTTTA  CCCACCTTGT  AAAAAATTAC  GAAAAATACA  ATAAAGTATT
4151  TTTAACAGGT  TATTATTATG  AAAAACATAA  AAAGCAGATT  AAAACTCAGT
4201  GCAATATCAA  TATTGCTTGG  CTTGGCTTCT  TCATCGACGT  ATGCAGAAGA
4251  AGCGTTTTTA  GTAAAAGGCT  TTCAGTTATC  TGGCGCG
```

FIG.9A.

```
   1  GGGAATGAGC GTCGTACACG GTACAGCAAC CATGCAAGTA GACGGCAATA
  51  AAACCACTAT CCGTAATAGC GTCAATGCTA TCATCAATTG GAAACAATTT
 101  AACATTGACC AAAATGAAAT GGAGCAGTTT TTACAAGAAA GCAGCAACTC
 151  TGCCGTTTTC AACCGTGTTA CATCTGACCA AATCTCCCAA TTAAAAGGGA
 201  TTTTAGATTC TAACGGACAA GTCTTTTTAA TCAACCCAAA TGGTATCACA
 251  ATAGGTAAAG ACGCAATTAT TAACACTAAT GGCTTTACTG CTTCTACGCT
 301  AGACATTTCT AACGAAAACA TCAAGGCGCG TAATTTCACC CTTGAGCAAA
 351  CCAAGGATAA AGCACTCGCT GAAATCGTGA ATCACGGTTT AATTACCGTT
 401  GGTAAAGACG GTAGCGTAAA CCTTATTGGT GGCAAAGTGA AAAACGAGGG
 451  CGTGATTAGC GTAAATGGCG GTAGTATTTC TTTACTTGCA GGGCAAAAAA
 501  TCACCATCAG CGATATAATA AATCCAACCA TCACTTACAG CATTGCTGCA
 551  CCTGAAAACG AAGCGATCAA TCTGGGCGAT ATTTTTGCCA AAGGTGGTAA
 601  CATTAATGTC CGCGCTGCCA CTATTCGCAA TAAAGGTAAA CTTTCTGCCG
 651  ACTCTGTAAG CAAAGATAAA AGTGGTAACA TTGTTCTCTC TGCCAAAGAA
 701  GGTGAAGCGG AAATTGGCGG TGTAATTTCC GCTCAAAATC AGCAAGCCAA
 751  AGGTGGTAAG TTGATGATTA CAGGTGATAA AGTCACATTA AAAACAGGTG
```

FIG.9B.

```
 801  CAGTTATCGA CCTTTCAGGT AAAGAAGGGG GAGAGACTTA TCTTGGCGGT
 851  GATGAGCGTG GCGAAGGTAA AAATGGTATT CAATTAGCGA AGAAAACCTC
 901  TTTAGAAAAA GGCTCGACAA TTAATGTATC AGGCAAAGAA AAAGGCGGGC
 951  GCGCTATTGT ATGGGGCGAT ATTGCATTAA TTAATGGTAA CATTAATGCT
1001  CAAGGTAGCG ATATTGCTAA AACTGGCGGC TTTGTGGAAA CATCAGGACA
1051  TGACTTATCC ATTGGTGATG ATGTGATTGT TGACGCTAAA GAGTGGTTAT
1101  TAGACCCAGA TGATGTGTCC ATTGAAACTC TTACATCTGG ACGCAATAAT
1151  ACCGGCGAAA ACCAAGGATA TACAACAGGA GATGGGACTA AAGAGTCACC
1201  TAAAGGTAAT AGTATTTCTA AACCTACATT AACAAACTCA ACTCTTGAGC
1251  AAATCCTAAG AAGAGGTTCT TATGTTAATA TCACTGCTAA TAATAGAATT
1301  TATGTTAATA GCTCCATCAA CTTATCTAAT GGCAGTTTAA CACTTCACAC
1351  TAAACGAGAT GGAGTTAAAA TTAACGGTGA TATTACCTCA AACGAAAATG
1401  GTAATTTAAC CATTAAAGCA GGCTCTTGGG TTGATGTTCA TAAAAACATC
1451  ACGCTTGGTA CGGGTTTTTT GAATATTGTC GCTGGGGATT CTGTAGCTTT
1501  TGAGAGAGAG GGCGATAAAG CACGTAACGC AACAGATGCT CAAATTACCG
1551  CACAAGGGAC GATAACCGTC AATAAAGATG ATAAACAATT TAGATTCAAT
1601  AATGTATCTA TTAACGGGAC GGGCAAGGGT TTAAAGTTTA TTGCAAATCA
```

FIG.9C.

```
1651  AAATAATTTC ACTCATAAAT TTGATGGCGA AATTAACATA TCTGGAATAG
1701  TAACAATTAA CCAAACCACG AAAAAAGATG TTAAATACTG GAATGCATCA
1751  AAAGACTCTT ACTGGAATGT TTCTTCTCTT ACTTTGAATA CGGTGCAAAA
1801  ATTTACCTTT ATAAAATTCG TTGATAGCGG CTCAAATTCC CAAGATTTGA
1851  GGTCATCACG TAGAAGTTTT GCAGGCGTAC ATTTTAACGG CATCGGAGGC
1901  AAAACAAACT TCAACATCGG AGCTAACGCA AAAGCCTTAT TTAAATTAAA
1951  ACCAAACGCC GCTACAGACC CAAAAAAAGA ATTACCTATT ACTTTTAACG
2001  CCAACATTAC AGCTACCGGT AACAGTGATA GCTCTGTGAT GTTTGACATA
2051  CACGCCAATC TTACCTCTAG AGCTGCCGGC ATAAACATGG ATTCAATTAA
2101  CATTACCGGC GGGCTTGACT TTTCCATAAC ATCCCATAAT CGCAATAGTA
2151  ATGCTTTTGA AATCAAAAAA GACTTAACTA TAAATGCAAC TGGCTCGAAT
2201  TTTAGTCTTA AGCAAACGAA AGATTCTTTT TATAATGAAT ACAGCAAACA
2251  CGCCATTAAC TCAAGTCATA ATCTAACCAT TCTTGGCGGC AATGTCACTC
2301  TAGGTGGGGA AAATTCAAGC AGTAGCATTA CGGGCAATAT CAATATCACC
2351  AATAAAGCAA ATGTTACATT ACAAGCTGAC ACCAGCAACA GCAACACAGG
2401  CTTGAAGAAA AGAACTCTAA CTCTTGGCAA TATATCTGTT GAGGGGAATT
```

FIG.9D.

```
2451  TAAGCCTAAC TGGTGCAAAT GCAAACATTG TCGGCAATCT TTCTATTGCA
2501  GAAGATTCCA CATTTAAAGG AGAAGCCAGT GACAACCTAA ACATCACCGG
2551  CACCTTTACC AACAACGGTA CCGCCAACAT TAATATAAAA CAAGGAGTGG
2601  TAAAACTCCA AGGCGATATT ATCAATAAAG GTGGTTTAAA TATCACTACT
2651  AACGCCTCAG GCACTCAAAA AACCATTATT AACGGAAATA TAACTAACGA
2701  AAAAGGCGAC TTAAACATCA AGAATATTAA AGCCGACGCC GAAATCCAAA
2751  TTGGCGGCAA TATCTCACAA AAAGAAGGCA ATCTCACAAT TTCTTCTGAT
2801  AAAGTAAATA TTACCAATCA GATAACAATC AAAGCAGGCG TTGAAGGGGG
2851  GCGTTCTGAT TCAAGTGAGG CAGAAAATGC TAACCTAACT ATTCAAACCA
2901  AAGAGTTAAA ATTGGCAGGA GACCTAAATA TTTCAGGCTT TAATAAAGCA
2951  GAAATTACAG CTAAAAATGG CAGTGATTTA ACTATTGGCA ATGCTAGCGG
3001  TGGTAATGCT GATGCTAAAA AAGTGACTTT TGACAAGGTT AAAGATTCAA
3051  AAATCTCGAC TGACGGTCAC AATGTAACAC TAAATAGCGA AGTGAAAACG
3101  TCTAATGGTA GTAGCAATGC TGGTAATGAT AACAGCACCG GTTTAACCAT
3151  TTCCGCAAAA GATGTAACGG TAAACAATAA CGTTACCTCC CACAAGACAA
3201  TAAATATCTC TGCCGCAGCA GGAAATGTAA CAACCAAAGA AGGCACAACT
3251  ATCAATGCAA CCACAGGCAG CGTGGAAGTA ACTGCTCAAA ATGGTACAAT
```

FIG.9E.

```
3301  TAAAGGCAAC ATTACCTCGC AAAATGTAAC AGTGACAGCA ACAGAAAATC
3351  TTGTTACCAC AGAGAATGCT GTCATTAATG CAACCAGCGG CACAGTAAAC
3401  ATTAGTACAA AAACAGGGGA TATTAAAGGT GGAATTGAAT CAACTTCCGG
3451  TAATGTAAAT ATTACAGCGA GCGGCAATAC ACTTAAGGTA AGTAATATCA
3501  CTGGTCAAGA TGTAACAGTA ACAGCGGATG CAGGAGCCTT GACAACTACA
3551  GCAGGCTCAA CCATTAGTGC GACAACAGGC AATGCAAATA TTACAACCAA
3601  AACAGGTGAT ATCAACGGTA AAGTTGAATC CAGCTCCGGC TCTGTAACAC
3651  TTGTTGCAAC TGGAGCAACT CTTGCTGTAG GTAATATTTC AGGTAACACT
3701  GTTACTATTA CTGCGGATAG CGGTAAATTA ACCTCCACAG TAGGTTCTAC
3751  AATTAATGGG ACTAATAGTG TAACCACCTC AAGCCAATCA GGCGATATTG
3801  AAGGTACAAT TTCTGGTAAT ACAGTAAATG TTACAGCAAG CACTGGTGAT
3851  TTAACTATTG GAAATAGTGC AAAAGTTGAA GCGAAAAATG GAGCTGCAAC
3901  CTTAACTGCT GAATCAGGCA AATTAACCAC CCAAACAGGC TCTAGCATTA
3951  CCTCAAGCAA TGGTCAGACA ACTCTTACAG CCAAGGATAG CAGTATCGCA
4001  GGAAACATTA ATGCTGCTAA TGTGACGTTA AATACCACAG GCACTTTAAC
4051  TACTACAGGG GATTCAAAGA TTAACGCAAC CAGTGGTACC TTAACAATCA
```

FIG.9F.

```
4101  ATGCAAAAGA TGCCAAATTA GATGGTGCTG CATCAGGTGA CCGCACAGTA
4151  GTAAATGCAA CTAACGCAAG TGGCTCTGGT AACGTGACTG CGAAAACCTC
4201  AAGCAGCGTG AATATCACCG GGGATTTAAA CACAATAAAT GGGTTAAATA
4251  TCATTTCGGA AAATGGTAGA AACACTGTGC GCTTAAGAGG CAAGGAAATT
4301  GATGTGAAAT ATATCCAACC AGGTGTAGCA AGCGTAGAAG AGGTAATTGA
4351  AGCGAAACGC GTCCTTGAGA AGGTAAAAGA TTTATCTGAT GAAGAAAGAG
4401  AAACACTAGC CAAACTTGGT GTAAGTGCTG TACGTTTCGT TGAGCCAAAT
4451  AATGCCATTA CGGTTAATAC ACAAAACGAG TTTACAACCA AACCATCAAG
4501  TCAAGTGACA ATTTCTGAAG GTAAGGCGTG TTTCTCAAGT GGTAATGGCG
4551  CACGAGTATG TACCAATGTT GCTGACGATG GACAGCAGTA GTCAGTAATT
4601  GACAAGGTAG ATTTCATCCT GCAATGAAGT CATTTTATTT TCGTATTATT
4651  TACTGTGTGG GTTAAAGTTC AGTACGGGCT TTACCCACCT TGTAAAAAAT
4701  TA
```

FIG.10A. COMPARISON OF DERIVED AMINO ACID SEQUENCE

```
            1                                                  50
Hmw3com     .......... .......... .......... .......... ..........
Hmw4com     .......... .......... .......... .......... ..........
Hmw1com     MNKIYRLKFS KRLNALVAVS ELARGCDHST EKGSEKPARM KVRHLALKPL
Hmw2com     MNKIYRLKFS KRLNALVAVS ELARGCDHST EKGSEKPARM KVRHLALKPL

            51                                                 100
Hmw3com     .......... .......... .......... .......... ..........
Hmw4com     .......... .......... ..GMSVVHGT ATMQVDGNKT TIRNSVNAII
Hmw1com     SAMLLSLGVT SIPQSVLASG LQGMSVVHGT ATMQVDGNKT TIRNSVNAII
Hmw2com     SAMLLSLGVT SIPQSVLASG LQGMSVVHGT ATMQVDGNKT TIRNSVNAII

            101                                                150
Hmw3com     .......... .......... .......... .......... ..........
Hmw4com     NWKQFNIDQN EMEQFLQESS NSAVFNRVTS DQISQLKGIL DSNGQVFLIN
```

FIG.10B.

```
Hmw1com   NWKQFNIDQN EMVQFLQENN NSAVFNRVTS NQISQLKGIL DSNGQVFLIN
Hmw2com   NWKQFNIDQN EMVQFLQENN NSAVFNRVTS NQISQLKGIL DSNGQVFLIN

          151                                                 200
Hmw3com   .......... .......... .......... .......... ..........
Hmw4com   PNGITIGKDA IINTNGFTAS TLDISNENIK ARNFTLEQTK DKALAEIVNH
Hmw1com   PNGITIGKDA IINTNGFTAS TLDISNENIK ARNFTLEQTK DKALAEIVNH
Hmw2com   PNGITIGKDA IINTNGFTAS TLDISNENIK ARNFTLEQTK DKALAEIVNH

          201                                                 250
Hmw3com   .......... .......... .......... .......... ..........
Hmw4com   GLITVGKDGS VNLIGGKVKN EGVISVNGGS ISLLAGQKIT ISDIINPTIT
Hmw1com   GLITVGKDGS VNLIGGKVKN EGVISVNGGS ISLLAGQKIT ISDIINPTIT
Hmw2com   GLITVGKDGS VNLIGGKVKN EGVISVNGGS ISLLAGQKIT ISDIINPTIT

          251                                                 300
Hmw3com   .......... INLGDIFAKG GNINVRAATI RNKGKLSADS VSKDKSGNIV
```

FIG.10C.

```
Hmw4com   YSIAAPENEA INLGDIFAKG GNINVRAATI RNKGKLSADS VSKDKSGNIV
Hmw1com   YSIAAPENEA VNLGDIFAKG GNINVRAATI RNKGKLSADS VSKDKSGNIV
Hmw2com   YSIAAPENEA VNLGDIFAKG GNINVRAATI RNKGKLSADS VSKDKSGNIV

                301                                          350
Hmw3com   LSAKEGEAEI GGVISAQNQQ AKGGKLMITG DKVTLKTGAV IDLSGKEGGE
Hmw4com   LSAKEGEAEI GGVISAQNQQ AKGGKLMITG DKVTLKTGAV IDLSGKEGGE
Hmw1com   LSAKEGEAEI GGVISAQNQQ AKGGKLMITG DKVTLKTGAV IDLSGKEGGE
Hmw2com   LSAKEGEAEI GGVISAQNQQ AKGGKLMITG DKVTLKTGAV IDLSGKEGGE

                351                                          400
Hmw3com   TYLGGDERGE GKNGIQLAKK TTLEKGSTIN VSGKEKGGRA IVWGDIALID
Hmw4com   TYLGGDERGE GKNGIQLAKK TTLEKGSTIN VSGKEKGGRA IVWGDIALID
Hmw1com   TYLGGDERGE GKNGIQLAKK TTLEKGSTIN VSGKEKGGRA IVWGDIALID
Hmw2com   TYLGGDERGE GKNGIQLAKK TTLEKGSTIN VSGKEKGGRA IVWGDIALID
```

FIG.10D.

```
          401                                                  450
Hmw3com   GNINAQGK.D IAKTGGFVET SGHYLSIDDN AIVKTKEWLL DPENVTIEAP
Hmw4com   GNINAQGS.D IAKTGGFVET SGHDLSIGDD VIVDAKEWLL DPDDVSIETL
Hmw1com   GNINAQGSGD IAKTGGFVET SGHDLFIKDN AIVDAKEWLL DPDNVTINAE
Hmw2com   GNINAQGSGD IAKTGGFVET SGHYLSIESN AIVKTKEWLL DPDDVTIEAE

          451                                                  500
Hmw3com   SASRVELGAD RNSHSAEVIK VTLKKNNTSL TTLTNTTISN LLKSAHVVNI
Hmw4com   TSGRNNTGEN QGYTTGDGTK ESPKGNSISK PTLTNSTLEQ ILRRGSYVNI
Hmw1com   TAGRSNTSED DEYTGSGNSA STPKRNKE.K TTLTNTTLES ILKKGTFVNI
Hmw2com   DPLRNNTGIN DEFPTGTGEA SDPKKNSELK TTLTNTTISN YLKNAWTMNI

          501                                                  550
Hmw3com   TARRKLTVNS SISIERGSHL ILHSEGQGGQ GVQIDKDITS .E...GGNLT
Hmw4com   TANNRIYVNS SINLSNGS.L TLHTK...RD GVKINGDITS NE...NGNLT
Hmw1com   TANQRIYVNS SINL.SNGSL TLWSEGRSGG GVEINNDITT GDDTRGANLT
Hmw2com   TASRKLTVNS SINGSNGSHL ILHSKGQRGG GVQIDGDIT. ...SKGGNLT
```

FIG.10E.

```
          551                                                  600
Hmw3com   IYSGGWVDVH KNITLGS.GF LNITTKEGDI AFEDKSGR.. ..NNLTITAQ
Hmw4com   IKAGSWVDVH KNITLGT.GF LNIVAGDS.V AFEREGDKAR NATDAQITAQ
Hmw1com   IYSGGWVDVH KNISLGAQGN INITAKQD.I AFEKGSNQV. ......ITGQ
Hmw2com   IYSGGWVDVH KNITLD.QGF LNITA.AS.V AFEGGNNKAR DANNLTITAQ

          601                                                  650
Hmw3com   GTITSG.NSN GFRFNNVSLN SLGGKLSFTD SREDRGRRTK GNISNKFDGT
Hmw4com   GTITVNKDDK QFRFNNVSIN GTGKGLKFIA NQN.......  .NFTHKFDGE
Hmw1com   GTIT.SGNQK GFRFNNVSLN GTGSGLQFTT KRTN.....K YAITNKFEGT
Hmw2com   GTVTITGEGK DFRANNVSLN GTGKGLNIIS SVNN......  ..LTHNLSGT

          651                                                  700
Hmw3com   LNISGTVDIS MKAPKVSWFY RD.KGRTYWN VTTLNVTSGS KFNLSIDSTG
Hmw4com   INISGIVTIN QTTKKDVKYW NA.SKDSYWN VSSLTLNTVQ KFTF.IKFVD
Hmw1com   LNISGKVNIS MVLPKNESGY DKFKGRTYWN LTSLNVSESG EFNLTIDSRG
```

FIG.10F.

```
Hmw2com   INISGNITIN QTTRKNTSYW QTSHD.SHWN VSALNLETGA NFTF.IKYIS

          701                                                 750
Hmw3com   SGSTG...PS IRNA..ELNG ITFN....KA TFNIAQGSTA NFSIKASIMP
Hmw4com   SGSNS...QD LRSSRRSFAG VHFNGIGGKT NFNIGANAKA LFKLKPNAAT
Hmw1com   SDSAGTLTQ. ....PYNLNG ISFN...KDT TFNVERNARV NFDIKAPIGI
Hmw2com   SNSKGLTTQY RSSAGVNFNG V..N...GNM SFNLKEGAKV NFKLKPNENM

          751                                                 800
Hmw3com   FKSNANYAL. FNEDISVSG. .GGSVNFKLN ASSSNIQTPG VIIKSQNFNV
Hmw4com   DPKKELPIT. FNANITATGN SDSSVMFDIH A...NLTSRA AGINMDSINI
Hmw1com   NKYSSLNYAS FNGNISVSG. .GGSVDFTLL ASSSNVQTPG VVINSKYFNV
Hmw2com   NTSKPLPI.R FLANITATG. .GGSVFFDIY ANHS...GRG AELKMSEINI

          801                                                 850
Hmw3com   SGGSTLNLKA EGSTETAFSI ENDLNLNATG GNITIRQVEG T..DSRVNKG
Hmw4com   TGGLDFSITS HNRNSNAFEI KKDLTINATG SNFSLKQTKD SFYNEYSKHA
```

FIG.10G.

```
Hmw1com   STGSSLRFKT SGSTKTGFSI EKDLTLNATG GNITLLQVEG T..DGMIGKG
Hmw2com   SNGANFTLNS HVRGDDAFKI NKDLTINATN SNFSLRQTKD DFYDGYARNA

                851                                          900
Hmw3com   VAAKKNITFK GGNITFGSQK ATTEIKGNVT INKNTNATLR GANFAEN...
Hmw4com   INSSHNLTIL GGNVTLGGEN SSSSITGNIN ITNKANVTLQ ADTSNSNTGL
Hmw1com   IVAKKNITFE GGNITFGSRK AVTEIEGNVT INNNANVTLI GSDFDNHQ..
Hmw2com   INSTYNISIL GGNVTLGGQN SSSSITGNIT IEKAANVTLE ANNAPNQQNI

                901                                          950
Hmw3com   KSPLNIAGNV INNGNLTTAG SIINIAGNLT VSKGANLQAI TNYTFNVAGS
Hmw4com   KKRTLTLGNI SVEGNLSLTG ANANIVGNLS IAEDSTFKGE ASDNLNITGT
Hmw1com   KPLTIKKDVI INSGNLTAGG NIVNIAGNLT VESNANFKAI TNFTFNVGGL
Hmw2com   RDRVIKLGSL LVNGSLSLTG ENADIKGNLT ISESATFKGK TRDTLNITGN

                951                                         1000
```

FIG.10H.

```
Hmw3com   FDNNGASNIS IARGGAKFK. DINNTSSLNI TTNSDTTYRT IIKGNISNKS
Hmw4com   FTNNGTANIN IKQGVVKLQG DINNKGGLNI TTNASGTQKT IINGNITNEK
Hmw1com   FDNKGNSNIS IAKGGARFK. DIDNSKNLSI TTNSSSTYRT IISGNITNKN
Hmw2com   FTNNGTAEIN ITQGVVKLG. NVTNDGDLNI TTHAKRNQRS IIGGDIINNK

                1001                                        1050
Hmw3com   GDLNIIDKKS DAEIQIGGNI SQKEGNLTIS SDKVNITNQI TIKAGVEGGR
Hmw4com   GDLNIKNIKA DAEIQIGGNI SQKEGNLTIS SDKVNITNQI TIKAGVEGGR
Hmw1com   GDLNITNEGS DTEMQIGGDI SQKEGNLTIS SDKINITKQI TIKAGVDGEN
Hmw2com   GSLNITDSNN DAEIQIGGNI SQKEGNLTIS SDKINITKQI TIKKGIDGED

                1051                                        1100
Hmw3com   SDSSEAENAN LTIQTKELKL AGDLNISGFN KAEITAKNGS DLTIGNASGG
Hmw4com   SDSSEAENAN LTIQTKELKL AGDLNISGFN KAEITAKNGS DLTIGNASGG
Hmw1com   SDSDATNNAN LTIKTKELKL TQDLNISGFN KAEITAKDGS DLTIGNTNSA
Hmw2com   SSSDATSNAN LTIKTKELKL TEDLSISGFN KAEITAKDGR DLTIGNSNDG
```

FIG.10I.

```
          1101                                                    1150
Hmw3com   N..ADAKKVT FDKVKDSKIS TDGHNVTLNS EVKT..SNGS SNAGNDNSTG
Hmw4com   N..ADAKKVT FDKVKDSKIS TDGHNVTLNS EVKT..SNGS SNAGNDNSTG
Hmw1com   D.GTNAKKVT FNQVKDSKIS ADGHKVTLHS KVETSGSNNN TEDSSDNNAG
Hmw2com   NSGAEAKKVT FNNVKDSKIS ADGHNVTLNS KVKTSSSNGG RESNSDNDTG

          1151                                                    1200
Hmw3com   LTISAKDVTV NNNVTSHKTI NISAAAGNVT TKEGTTINAT TGSVEVTAQN
Hmw4com   LTISAKDVTV NNNVTSHKTI NISAAAGNVT TKEGTTINAT TGSVEVTAQN
Hmw1com   LTIDAKNVTV NNNITSHKAV SISATSGEIT TKTGTTINAT TGNVEIT...
Hmw2com   LTITAKNVEV NKDVTSLKTV NITA.SEKVT TTAGSTINAT NGKASIT...

          1201                                                    1250
Hmw3com   GTIKGNITSQ NVTVTATENL VTTENAVINA TSGTVNISTK TGDIKGGIES
Hmw4com   GTIKGNITSQ NVTVTATENL VTTENAVINA TSGTVNISTK TGDIKGGIES
Hmw1com   .......... .......... .......... ........AQ TGDIKGGIES
```

FIG.10J.

```
Hmw2com  ..........  ..........  ..........  ........TK T.........

          1251                                                1300
Hmw3com  TSGNVNITAS GNTLKVSNIT GQDVTVTADA GALTTTAGST ISATTGNANI
Hmw4com  TSGNVNITAS GNTLKVSNIT GQDVTVTADA GALTTTAGST ISATTGNANI
Hmw1com  SSGSVTLTAT EGALAVSNIS GNTVTVTANS GALTTLAGST IKG.TESVTT
Hmw2com  ..........  ..........  ..........  ..........  ..........

          1301                                                1350
Hmw3com  TTKTGDINGK VESSSGSVTL VATGATLAVG NISGNTVTIT ADSGKLTSTV
Hmw4com  TTKTGDINGK VESSSGSVTL VATGATLAVG NISGNTVTIT ADSGKLTSTV
Hmw1com  SSQSGDIG..  ..........  .........G TISGGTVEVK ATESLTTQSN
Hmw2com  ....GDIS..  ..........  .........G TISGNTVSVS ATVDLTTKSG

          1351                                                1400
Hmw3com  GSTINGTNSV TTSSQSGDIE GTISGNTVNV TASTGDLTIG NSAKVEAKNG
Hmw4com  GSTINGTNSV TTSSQSGDIE GTISGNTVNV TASTGDLTIG NSAKVEAKNG
```

FIG.10K.

```
Hmw1com   SKIKATTGEA NVTSATGTIG GTISGNTVNV TANAGDLTVG NGAEINATEG
Hmw2com   SKIEAKSGEA NVTSATGTIG GTISGNTVNV TANAGDLTVG NGAEINATEG

                1401                                        1450
Hmw3com   AATLTAESGK LTTQTGSSIT SSNGQTTLTA KDSSIAGNIN AANVTLNTTG
Hmw4com   AATLTAESGK LTTQTGSSIT SSNGQTTLTA KDSSIAGNIN AANVTLNTTG
Hmw1com   AATLTTSSGK LTTEASSHIT SAKGQVNLSA QDSSVAGSIN AANVTLNTTG
Hmw2com   AATLTATGNT LTTEAGSSIT STKGQVDLLA QNSSIAGNIN AANVTLNTTG

                1451                                        1500
Hmw3com   TLTTTGDSKI NATSGTLTIN AKDAKLDGAA SGDRTVVNAT NASGSGNVTA
Hmw4com   TLTTTGDSKI NATSGTLTIN AKDAKLDGAA SGDRTVVNAT NASGSGNVTA
Hmw1com   TLTTVKGSNI NATSGTLTIN AKDAELNGAA LGNHTVVNAT NANGSGSVIA
Hmw2com   TLTTVAGSDI KATSGTLTIN AKDAKLNGDA SGDSTEVNAV NASGSGSVTA

                1501                                        1550
```

FIG.10L.

```
Hmw3com   KTSSSVNITG LLNTINGLNI ISENGRNTVR LRGKEIDVKY IQPGVASVEE
Hmw4com   KTSSSVNITG DLNTINGLNI ISENGRNTVR LRGKEIDVKY IQPGVASVEE
Hmw1com   TTSSRVNITG DLITINGLNI ISKNGINTVL LKGVKIDVKY IQPGIASVDE
Hmw2com   ATSSSVNITG DLNTVNGLNI ISKDGRNTVR LRGKEIEVKY IQPGVASVEE

          1551                                              1600
Hmw3com   VIEAKRVLEK VKDLSDEERE TLAKLGVSAV RFVEPNNAIT VNTQNEFTTK
Hmw4com   VIEAKRVLEK VKDLSDEERE TLAKLGVSAV RFVEPNNAIT VNTQNEFTTK
Hmw1com   VIEAKRILEK VKDLSDEERE ALAKLGVSAV RFIEPNNTIT VDTQNEFATR
Hmw2com   VIEAKRVLEK VKDLSDEERE TLAKLGVSAV RFVEPNNTIT VNTQNEFTTR

          1601                                  1632
Hmw3com   PSSQVTISEG KACFSSGNGA RVCTNVADDG QQ
Hmw4com   PSSQVTISEG KACFSSGNGA RVCTNVADDG QQ
Hmw1com   PLSRIVISEG RACFSNSDGA TVCVNIADNG R.
Hmw2com   PSSQVIISEG KACFSSGNGA RVCTNVADDG QP
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/02166

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :C07K 13/00, 15/04, 17/02; C07H 21/04; C12N 15/09, 15/31; A61K 39/02
US CL :530/350, 825; 536/27; 424/88, 92; 435/69.3
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350, 825; 536/27; 424/88, 92; 435/69.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, APS, IG SUITE
search terms: high molecular weight protein, haemophilus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	The Journal of Infectious Diseases, Volume 165(Suppl.), issued August 1992, S.J.Barenkamp., "Outer Membrane Protein and Lipopolysaccharides of Nontypeable *Haemophilus influenzae*", pages S181-S184, see entire document.	1-19
Y,P	Infection and Immunity, Volume 60(4), issued April 1992, S.J.Barenkamp et al, "Cloning, Expression and DNA Sequence Analysis of Genes Encoding Nontypeable *Haemophilus influenzae* High-Molecular-Weight Surface-Exposed Proteins Related to Filamentous Hemagglutinin of *Bordetella pertussis*, pages 1302-1313, see entire document.	1-19

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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Date of the actual completion of the international search
14 May 1993

Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/02166

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Infection and Immunity, Volume 56(1), issued January 1988, E.J.Hansen, "Immune Enhancement of Pulmonary Clearance on Nontypable *Haemophilus influenzae*, pages 182-190, see entire document, especially Figures 3 and 4.	1-19
Y	Infection and Immunity, Volume 52(2), issued May 1986, S.J.Barenkamp, "Protection by Serum Antibodies in Experimental Nontypable *Haemophilus influenzae* Otitis Media", pages 572-578, see Figures 1 and 2.	1-19
Y	Proceedings of the National Academy of Sciences USA, Volume 80, issued March 1983, R.A.Young et al, "Efficient Isolation of Genes by Using Antibody Probes", pages 1194-1198, see entire document.	1-19
Y	Infection and Immunity, Volume 45(3), issued September 1984, R. Schneerson et al, "Serum Antibody Responses of Juvenile and Infant Rhesus Monkeys Injected with *Haemophilus influenzae* Type b and Pneumococcus Type 6A Capsular Polysaccharide-Protein Conjugates", pages 582-591, see entire document.	16-17
Y	Journal of Molecular Biology, Volume 157, issued 1982, J.Kyte et al, "A Simple Method for Displaying the Hydropathic Character of a Protein", pages 105-132, see entire document.	18-19
Y	Proceedings of the National Academy of Sciences, Volume 78(6), issued June 1981, T.P.Hopp et al, "Prediction of Protein Antigenic Determinants from Amino Acid Sequences", pages 3824-3828, see entire document.	18-19